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

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List of abbreviations: AD: acute decompensation, ACLF: acute-on chronic liver failure, CSI: clinically significant bacterial infections, FCN: ficolin, MASP: mannan-binding lectin serine proteases, MBL: mannan-binding lectin, MELD: model for end-stage liver disease, HBV: hepatitis B virus, HCV: hepatitis C virus, SBP: spontaneous bacterial peritonitis, SNP: single nucleotide 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 (<427ng/ml, *pLogRank*=0.047) and FCN-3 (<4857ng/ml, *pLogRank*=0.029), but not with MASP-2 deficiency (<100ng/ml, *pLogRank*=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%, *pLogRank*=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

constituent of cirrhosis-associated immune dysfunction.

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Key words: ficolin, mannan-binding lectin serine protease, cirrhosis, bacterial infection, mortality

Key Points

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

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

[13]. Low levels of the functional proteins increase the risk of various infectious diseases mostly in immune-deficient conditions [14–16]. Association of deficiencies in lectin pathway molecules with the development of infectious episodes in patients with cirrhosis is quite reasonable, but has scarcely been investigated. Our group has previously demonstrated, that the absolute MBL deficiency is a risk for the development of clinically significant bacterial infections (CSI) independently from both the disease severity and the presence of co-morbidity. Furthermore, in a small study of *Chong et al.*, the *MBL2* deficient genotype predisposed patients with hepatitis B virus(HBV) induced liver cirrhosis to develop SBP [17].

In the present study, we aimed to assess the serum levels of various lectin pathway molecules in a large cohort of stable outpatients with cirrhosis and also their association with the disease specific characteristics. Additionally, in a 5-year follow-up observational study we aimed to evaluate whether serum levels of various lectin pathway molecules constitute a risk for the development of cirrhosis-associated bacterial infections.

PATIENTS AND METHODS

Patient population

We performed a cohort study among adult patients with established diagnosis of cirrhosis of different etiologies in a tertiary care referral center of Hungary (Division of Gastroenterology Department of Internal Medicine, Clinical Center, University of Debrecen). The present study population is a part of our entire patient cohort comprising a total of 404 patients with cirrhosis and recruited consecutively between May 1, 2006 and December 31, 2010 from

the outpatient clinic during regular or extraordinary follow-up visits and also from the inpatient ward owing to hospitalization with an acute decompensation (AD) episode [18]. In this study only stable outpatients with available serum samples (n=266) were included.

Clinical characteristics of patients at inclusion are presented in **Table 1**. Blood samples, routine laboratory data and detailed clinical phenotype were captured at inclusion. Clinical data were determined by in-depth review of the patients' medical records using a structured interview. Medical records that documented age at diagnosis, etiology, presence of hepatocellular carcinoma, esophageal varices, extrahepatic co-morbidities, history of previous AD episode(s), and cirrhosis-related medication were retrospectively analyzed for the period prior to the observational follow-up study. At enrolment, disease severity assessed by liver-oriented scores (Child-Pugh and MELD) was determined.

Phenotypical characterization of patients during follow-up

Patients were enrolled into an observational follow-up study, where the attending gastroenterologist registered date and type of clinically significant bacterial infections (CSI) warranted hospital admission. Follow-up period lasted 5 years, or until death/loss of follow-up. The median follow-up was 979 days (IQR: 331-1825). Collected data were transferred and stored in a database. At the end of the study period, December 31, 2013, all clinical data were extracted for further analysis.

Development of CSI was carefully established by compatible clinical symptoms and findings, laboratory data (leucocyte count, high-sensitivity C-

reactive protein and procalcitonin, results of urine analysis (sediment) and imaging findings (abdominal ultrasound and chest X-ray) and if ascites was present the result of diagnostic tap (neutrophil count and ascites culture) was considered. Based on the results of this procedure, cultures from specific sites (sputum, urine, wound discharge, etc.) were obtained according to location of infection, though blood cultures were obtained in sepsis, or if the location of the infection could not be clearly identified. Regarding laboratory results, elevated leucocyte count (absolute >10.8 G/L or relative [in patients with leukopenia]: double of count at former visits) with an elevated neutrophil rate (>76%) and increased serum levels of CRP (>10.0 mg/L) and/or PCT (>0.15 µg/L) [19] were considered to support the diagnosis of infection. Based on specific clinical symptoms and findings the following infections were diagnosed. Further characterization of bacterial infections was done on the basis of conventional criteria [20–23]. (1) Spontaneous bacterial peritonitis: neutrophil cell count >250/mm3 and/or positive culture of ascitic fluid, in the absence of intra-abdominal source of infection. (2) Urinary tract infection: presence of dysuria, pyuria (leucocyte >10/mm3) and positive urine culture. (3) Pneumonia: presence of cough and expectoration, positive chest X-ray, positive sputum culture. (4) Miscellaneous: skin and soft tissue, biliary tract, orocavital, intestinal tract infection, osteomyelitis, endocarditis. (5) Bacterial infection with unknown origin: positive blood culture in the absence of site-specific infection.

The healthy control group consisted of 160 age- and gender-matched individuals (male/female: 72/88, age: 51.5±16.9 years) selected from consecutive blood donors in Debrecen. The control subjects did not have any

known gastrointestinal or liver diseases.

Serological analysis

Blood samples were obtained at enrolment from each patient and were frozen at -70°C until testing collectively after end of the study period.

Serum level of MASP-2 was determined by a commercially available solid-phase enzyme-linked immunoassay(ELISA), according to the manufacturer's instructions (Hycult Biotechnology, Uden, Netherlands). MASP-2 deficiency was defined a level of <100ng/ml in agreement with literature data [24].

Serum level of L-ficolin (Liver ficolin, ficolin-2 [FCN-2]) and H-ficolin, Hakata ficolin, ficolin-3 [FCN-3]) were determined by an in-house developed double-antibody sandwich ELISA. Since no definition exists for FCN deficiency, low level of FCN-2 (<427ng/ml) and FCN-3 (<4857ng/ml) were determined arbitrarily corresponding to the 25th serum level percentile of the patients.

Detailed description of these experiments such as analytical properties are presented in the **Supplementary Material**.

Ethical considerations

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

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

Serum levels of lectin pathway molecules in patients with cirrhosis and the control group are presented in **Table 2**. FCN-2, FCN-3 and MASP-2 levels were significantly lower in patients with cirrhosis compared to healthy subjects. Similarly, MASP-2 deficiency occurred more often in patients with cirrhosis.

In cirrhosis, levels of all three lectin pathway molecules decreased gradually according to disease severity, as rated by the Child-Pugh stage. Furthermore, both types of the FCNs but not the MASP-2 levels were significantly lower in patients with ascites as compared to those without (**Table 2**).

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

Significant correlation was found between levels of lectin pathway molecules and laboratory markers of impaired renal and liver function and accordingly with liver-oriented scores (Child-Pugh and MELD). Non-parametric correlations are summarized in **Table 3**.

Significance of serum levels of lectin pathway molecules in the risk of clinically significant bacterial infections

Ninety-five (35.7%) of involved patients encountered at least one episode of CSI during the follow-up period. The median time to the development of first

infectious episode was 626 [169-799] days. Urinary tract infection was the most commonly diagnosed CSI and accounted for 33.7% of events, followed by SBP (24.2%) and pneumonia (12.6%). 5.3% of the cases were multifocal. Other sites of bacterial infections were as follows: erysipelas (4.2%), cholangitis (2.1%), bacteremia (2.1%), acute bronchitis (3.2%) and unidentified in 12 (12.6%) cases.

Low FCN-2 and FCN-3 levels were associated with an increased cumulative probability of CSI compared to normal level of the molecules (62.6% vs. 46.7% for FCN-2 with HR: 1.55, 95%CI: 1.00-2.39, p=0.047 and 59.3% vs. 48.2% for FCN-3 with HR: 1.61, 95%CI: 1.05-2.47, p=0.029) (**Table 4**). FCN serum profile, considering both levels of FCN molecules, showed an ever-increasing cumulative risk for development of CSI with increasing number of low FCN levels. Patients with normal level of both FCNs had a cumulative probability of 45.7% to develop a CSI, while those with low level of one or two FCNs had 57.2% and 63.8%, respectively (HR [95%CI]: 1.39 [0.87-2.22], p=0.164 and 2.00 [1.15-3.47], p=0.016, respectively) (**Table 4** and **Figure 1A**). No similar association was found, however, in the case of MASP-2 deficiency either individually (**Table 4** and **Figure 1B**) or in combination with FCN levels (data not shown).

Covariates

Analysis of clinical factors associated with development of CSI, using univariate Cox regression analysis, is shown in **Table 4**. Alcoholic disease etiology (HR: 1.68, 95%CI: 1.07-2.66, p=0.024), advanced disease stage (Child-Pugh stage B/C [HR: 2.42, 95%CI: 1.61-3.64, p<0.001] or presence of

ascites [HR: 2.31, 95%CI: 1.54-3.46, p<0.001]) and prior CSI episode (HR: 2.64, 95%CI: 1.76-3.96, p<0.001) were significantly associated with the increased risk for the development of CSI.

Multivariate analysis

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

Mortality

Potential impact of FCN serum profile and MASP-2 deficiency on infection-related mortality was also evaluated (n=95). During the course of the first bacterial infection, 19 patients died (20%). Neither FCN serum profile nor MASP-2 deficiency at enrolment was associated with the risk of mortality during a subsequent bacterial infection in Kaplan-Meier survival analysis (**Figure 1C and D**).

In the total cohort liver-related mortality occurred in 85 (32.0%) subjects. Kaplan-Meier survival analysis demonstrated a significantly worse survival in patients with advanced disease according to Child-Pugh stage (p<0.001) or ascites (p<0.001) or prior CSI episode (p=0.035). MASP-2 deficiency (p=0.387) or FCN serum profile (p=0.093), however, were not associated with overall survival (**Figure 1E and F**).

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 methology 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 *Laursen 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

(n=120) with chronic HBV infection related cirrhosis by *Hoang et al*. [29]. Contrary, in a single study of patients with chronic HCV infection [30], serum FCN-2 levels were significantly higher in patients with cirrhosis (n=21) than those in the control and other patient groups including chronic inactive or active HCV patients without cirrhosis. The reason, why serum levels of lectin pathway molecules are decreased in most of the patients with cirrhosis is yet to be elucidated. One reasonable explanation could be that in cirrhosis, synthetic capacity of hepatocytes is damaged, that results in a decreased production of these proteins. This assumption is supported by our findings that FCN-2, FCN-3 and MASP-2 levels showed gradual decrease according to disease severity.

When the adaptive immune response is immature or compromised, the innate immune system constitutes the principle defense against infection. Clinical studies have shown that common functional gen polymorphisms in *FNC2* [27], *FCN3* [16] and *MASP2* [15], which affect the composition, structure and function of respective proteins, confer an increased risk for various bacterial infections particularly in immunocompromised patients. Cirrhosis has been characterized as an acquired immunodeficiency syndrome [31]. Thus, it is reasonable to assume that serum levels of the lectin pathway molecules might be related to the bacterial infectious episodes among patients with cirrhosis; however, it has not been evaluated so far. Accordingly, we conducted a follow-up observational study to assess whether the low level of FCNs or MASP-2 deficiency constitutes a risk for the development of CSI in patients with cirrhosis.

Several SNPs in the promoter and the encoding region of *FCN2* and

MASP2 gene have been implicated in influencing protein concentrations but relatively few SNP has been identified in *FCN3* gene [15,16]. Considerable linkage disequilibrium occurs between pairs of promoter and structural gene dimorphisms, confounding the investigation of the relationship between allele expression and protein concentration [27]. Thus it has been clearly proved, that genotypes have very modest influence on average protein concentrations, each being associated with large and overlapping ranges [32]. Besides gene polymorphisms of the lectin pathway of complement system, up- and downregulation of these genes and increase or decrease of serum proteins also appears to be associated with various infectious and inflammatory diseases [16]. Due to these limitations we evaluated serum level of the molecules instead of genotyping.

Regarding serological markers, however one could take into consideration various factors, that might influence stability of the serum level of molecule. Acute phase reaction might be such a factor, thus to avoid interfering effect of acute complication of the disease on the synthesis of the molecules, only stable outpatients were involved in the present study. Moreover, in a subgroup of patients (n=32) we carefully evaluated whether the level of FCN-2, FCN-3 and MASP-2 are stable over time. They have a subsequent sample from a later outpatient visit with time-lag of 655 [IQR: 246-1090] days. Median time between sample procurements was comparable with the time to first CSI episode. None of the serum level of these molecules showed significant changes (FCN-2: 504 [458-600] vs. 479 [423-543], p=0.092; FCN-3: 9449 [5934-13070] vs. 8814 [5999-11006], p=0.837; MASP-2: 221 [129-369] vs. 208 [119-324], p=0.313). No significant change in

patients' Child-Pugh category was observed during this time period (p=1.000 with Wilcoxon signed ranks test).

Low level of sPRMs can lead to insufficient recognition of microbes and decreased complement activation, opsonization and inflammatory cytokine secretion, which results in increased susceptibility to infections [33]. In Kaplan-Meier analysis, in patients with low level of either FCN-2 or FCN-3 the cumulative probability of CSI was significantly higher, compared to those with normal level. Moreover, considering both levels of FCN molecules a step-wise increase was observed in the risk of the CSI development with increasing number of low FCN levels. In the multivariate Cox-regression analysis, adjusting for Child-Pugh category, however, these associations were not found to be independent from diseases severity. This might support the notion, that decreased level of FCNs are related to deterioration of synthetic capacity of hepatocytes. And also suggests that low level of FCNs is an additional constituent of cirrhosis-associated immune deficiency and increased risk of CSI in this patient population.

MASP-2 is the central but not a single activator of the lectin pathway. After lectin type sPRMs bind their targets, MASPs are cleaved and activated and set in motion a proteolytic cascade, which culminates in formation of the membrane attack complex and pathogen lysis [15]. In the present study, serum MASP-2 level was not associated with the risk of developing a CSI. However, we did not perform functional analysis that is an indisputable limitation of our study. MASP-2 antigen level not necessarily indicates the function of the molecule and scarcely assessed in previous studies. One cannot exclude that MASP-2 activity could have been associated with this

outcome.

Finally, serum level of lectin pathway components at enrolment was also not associated with the infection-related mortality. Since complement system has a dual role in disease susceptibility, one can expect that during an on-going inflammatory process rather its excessive activation with subsequent tissue damage than its lack of activity poses a risk of harm to the host. Another important consideration is that serum level of these molecules might change during acute phase reactions like bacterial infection. We did not re-assess serum samples during the first CSI episodes, which is evidently another limitation of this analysis. These issues warrant further evaluation in future studies of the lectin pathway in cirrhosis.

In conclusion, findings of our large referral cohort study indicate that serum level of the lectin complement pathway molecules are decreased in cirrhosis and are related to the disease severity verifying importance of the liver in the production of FCNs and MASP-2. Moreover, low levels of soluble pattern recognition FCN molecules might be considered as an additional constituent of cirrhosis-associated immune dysfunction resulting in an increased risk of bacterial infections in this patient population.

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; [a]median, 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; [a]median, 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

[‡]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

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		.	.	Univariate analysis	.	Mulivariate analysis	.
.	.	n of subject	n of event	CP of CSI %	p-value*	HR (95% CI)	p-value	HR (95% CI)	p-value
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

* p-values of the log-rank tests;

CP: cumulative probability; CI: confidence interval; CSI: clinically significant bacterial infections; FCN: ficolin; HR: hazard ratio; MASP: mannan-binding lectin serine protease; NSBB: non-selective beta blocker; PPI: proton-pump inhibitor; ref.: reference

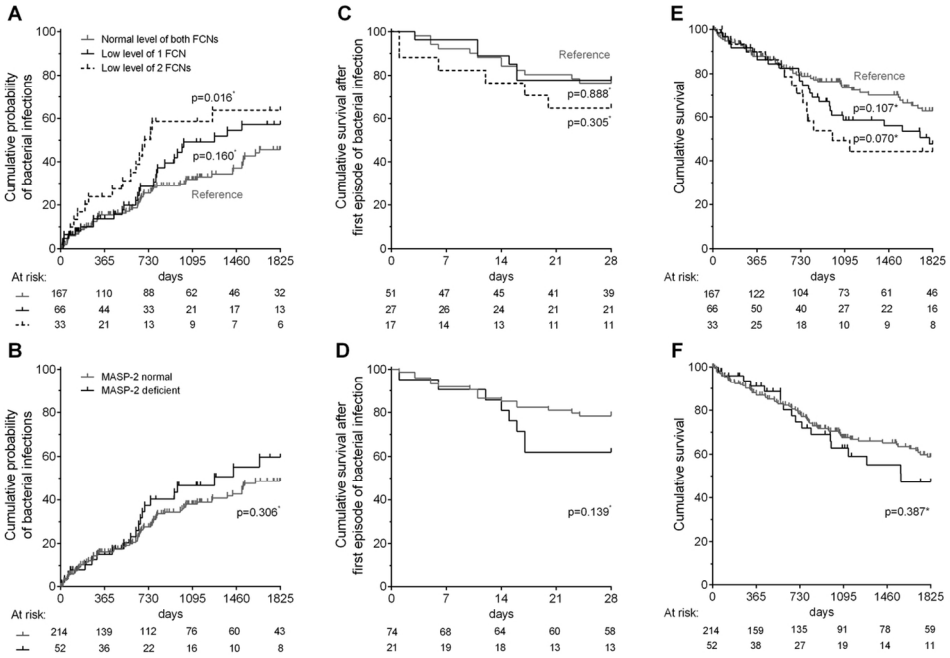

Figure 1
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