Research Article

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Mannose-binding lectin deficiency confers risk for bacterial infections in a large Hungarian cohort of patients with liver cirrhosis☆

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Background & Aims: Mannose-binding lectin (MBL) is a serum lectin synthesized by the liver and involved in innate host defense. MBL deficiency increases the risk of various infectious diseases mostly in immune-deficient conditions. Bacterial infections are a significant cause of morbidity and mortality in liver cirrhosis due to the relative immuncompromised state.

Methods: Sera of 929 patients with various chronic liver diseases [autoimmune liver diseases (ALD), 406; viral hepatitis C (HCV), 185; and liver cirrhosis (LC) with various etiologies, 338] and 296 healthy controls (HC) were assayed for MBL concentration. Furthermore, a follow-up, observational study was conducted to assess MBL level as a risk factor for clinically significant bacterial infections in cirrhotic patients.

Results: MBL level and the prevalence of absolute MBL deficiency (<100 ng/ml) was not significantly different between patients and controls (ALD: 14.5%, HCV: 11.9%, LC: 10.7%, HC: 15.6%). In cirrhotic patients, the risk for infection was significantly higher among MBL deficient subjects as compared to non-deficient ones (50.0% vs. 31.8%, $p = 0.039$). In a logistic regression analysis, MBL deficiency was an independent risk factor for infections (OR: 2.14 95% CI: 1.03–4.45, $p = 0.04$) after adjusting for Child–Pugh score, co-morbidities, gender, and age. In a Kaplan–Meier analysis, MBL deficiency was associated with a shorter time to develop the first infectious complication (median days: 579 vs. 944, *pBreslow* = 0.016, *pLogRank* = 0.027) and was identified as an independent predictor in a multivariate Cox-regression analysis ($p = 0.003$, OR: 2.33, 95% CI: 1.34–4.03).

Conclusions: MBL deficiency is associated with a higher probability and shorter time of developing infections in liver cirrhosis, further supporting the impact of the MBL molecule on the host defense.

Introduction

Bacterial infections are a common cause of morbidity in patients with liver cirrhosis. A wide range of bacterial infections can decompensate hepatic status and lead to death in cirrhotic patients [1,2]. Moreover, bacterial infections have been acknowledged as a potential trigger factor in many complications of liver cirrhosis, including variceal bleeding, hepatic encephalopathy, renal failure, and impairment in clotting factors [3]. Multiple levels of immune dysfunction have been found in cirrhotic patients rendering them susceptible to bacterial infections [4], marked depression of reticuloendothelial systemic function, dysfunction of polymorphonuclear leukocytes, or the deficient complement system. Advanced disease, as reflected by the Child–Pugh stage [5,6], and the presence of gastrointestinal hemorrhage [7,8] are independent predictors of bacterial infections in patients with liver cirrhosis. Regardless of the severity of the hepatic insufficiency, the development of the infection significantly increases the mortality rate. The in-hospital mortality of cirrhotic patients with infection is more than twice that of patients without infection. Various infections are directly responsible for 30–50% of death in liver cirrhosis [1,6].

Keywords: Mannose-binding lectin; Chronic liver diseases; Liver cirrhosis; Bacterial infection.

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Abbreviations: MBL, mannose-binding lectin; ALD, autoimmune liver diseases; PBC, primary biliary cirrhosis; AIH, autoimmune hepatitis; HCV, hepatitis C virus; AMA, anti-mitochondrial antibodies; MELD, model for end-stage liver disease; SD, standard deviation; IQR, interquartile range or range; CI, confidence interval.

Mannose-binding lectin (MBL) is a major pattern-recognition molecule and an important component of the innate immune system. The molecule is able to recognize a wide range of common pathogens through their surface carbohydrate sequences and eradicate them. Upon binding to pathogens, MBL in a complex with MBL-associated serin protease-2 mediates direct opsonophagocytosis and stimulates complement activation via the lectin pathway. The molecule is synthesized mainly in the liver and is secreted into the bloodstream [9]. Large inter-individual variations in serum MBL levels are well-documented in healthy persons, but individual values are very stable over time, largely due to their genetic disposition [10,11]. The serum levels of functional MBL correlate well with the underlying *MBL2* coding genotype. Up to 40% of Caucasians were found to have low MBL levels, and up to 8% were found to have considerably reduced levels (MBL deficient) [12] as a result of impaired assembly or stability of multimers [13].

MBL deficiency has been reported as a risk factor for infections [14,15], particularly when immunity is already compromised through immunological immaturity [16], co-morbidity, or medical therapy [17]. A significant correlation was reported between the risk of clinically significant post-transplant infections and the donor MBL deficiency defined by the *MBL2* genotype in patients undergoing orthotopic liver transplantation. The post-transplantation serum MBL level was predicted by the hepatic and not the extrahepatic genotype [18,19]. In another study, a donor *MBL2* variant genotype significantly influenced the outcome of the liver transplantation, mainly because of infectious events of higher severity [20]. Finally, in a small study, the *MBL2* deficient genotype predisposed patients with hepatitis B virus induced liver cirrhosis to develop spontaneous bacterial peritonitis [21].

In the present study, we aimed to assess serum MBL levels and the prevalence of MBL deficiency in a large cohort of chronic liver diseased patients with various etiologies and levels of cirrhosis. Additionally, we conducted a follow-up observational study to determine whether the MBL deficiency constitutes a risk factor for clinically significant bacterial infection in liver cirrhosis.

Patients and methods

Patients

Five-hundred-ninety-one patients with various chronic liver diseases were investigated. Sera of patients with autoimmune liver diseases [ALD] ($n = 406$, male/female ratio [m/f]: 122/184, age: 50.5 ± 17.0 years [yrs]: primary biliary cirrhosis [PBC, $n = 182$], primary sclerosing cholangitis [PSC, $n = 76$], and autoimmune hepatitis [$n = 148$]) or chronic hepatitis C (chronic HCV, $n = 185$, m/f: 90/95, age: 54.3 ± 12.5 yrs) were collected from six Hepatology Centers, five Hungarian (Debrecen University, Budapest Semmelweis University, Pecs University, Miskolc Borsod-Abauj Zemplen County Hospital and Miskolc Szent Ferenc Hospital) and one German (Otto-von-Guericke University, Magdeburg) (Table 1). The diagnosis of PBC was based on biochemical evidence of cholestasis, serum anti-mitochondrial antibodies (AMA) and/or PBC-specific AMA-M2 positivity, compatible histology, and with the exclusion of extrahepatic cholestasis [22]. The diagnosis of PSC was based on biochemical evidence of cholestasis and the characteristic cholangiographic findings of bile duct stenoses and dilatations. In most cases, diagnosis was confirmed by compatible histology findings [23]. The diagnosis of AIH was based on the exclusion of other major causes of liver damage, including alcoholic, viral, drug and toxin-induced, and hereditary liver disease, and by using the scoring system of the International AIH Group [24]. The diagnosis of chronic HCV was based on positive HCV RNA, elevated liver function tests (>2× ULN for more than 6 months) and compatible liver biopsy, if available. The central coordination of sample and database management was done by the Gastroenterology Division of 2nd Department of Medicine, Debrecen University (M.P. and I.T.). The control group consisted of 296 age- and gender-matched healthy individuals (m/f: 140/156, age: 49.5 ± 16.5 yrs) selected from consecutive blood donors in Debrecen and Budapest. Control subjects did not have any gastrointestinal or liver diseases.

Serum samples were also obtained from 338 consecutive cirrhotic patients with various etiologies (m/f: 189/149, age: 56.4 ± 10.8 yrs) at the Gastroenterology Division of 2nd Department of Medicine (Debrecen University) during the period from May 2006 to April 2008. The clinical data of the cirrhotic patients are summarized in Table 2. The etiology of cirrhosis was alcoholic in 220 (65.1%), hepatitis C virus-related in 98 (29.0%) and other remaining etiologies in 20 (5.9%). The exclusion criteria were: evidence of gastrointestinal bleeding or bacterial infection in the preceding 6 weeks, and prophylactic treatment with non-absorbable antibiotics in the preceding 6 months. The diagnosis of cirrhosis was based on clinical, biochemical, ultrasonographic, and, when available, histological features. Clinical data, including age, age at onset, etiology and severity of cirrhosis, presence and grade of ascites, encephalopathy, esophageal varices, prior episode(s) of variceal bleeding and co-morbidities were collected. Myocardial infarction, congestive heart failure, peripheral arterial disease, cerebrovascular disease, chronic pulmonary disease, chronic renal failure, ulcer disease, diabetes mellitus, and non-metastatic and metastatic cancer, including hepatocellular carcinoma, were the co-morbidity diagnoses taken into account during data collection. The severity of the cirrhosis was graded according to the Child–Pugh classification [25], and the model for end-stage liver disease (MELD) score [26] was also calculated. Patients were involved afterwards in a follow-up study until April 1st, 2009 or death/loss of follow-up (median follow-up: 420 days [IQR: 87–745]) assessing the occurrence of clinically significant bacterial infections. Infectious episodes were identified from the hospital inpatient medical records ($n = 719$) reviewed for information on clinical symptoms, appearance of fever, and laboratory data, including microbiological culture results, if available, compatible findings of imaging techniques, and also the effect of antibiotic treatment by two independent gastroenterologists (M.P. and Zs.V.). Autopsy records ($n = 108$) were also assessed in case of death. Infections of skin and soft tissue, orocavital region, upper and lower respiratory tract (acute bronchitis, pneumonia), biliary tract (cholecystitis, cholangitis, liver abscess), intestinal tract (gastroenteritis), urinary tract (cystitis, pyelonephritis), osteomyelitis and endocarditis were diagnosed on the basis of conventional criteria. The diagnosis of spontaneous bacterial peritonitis was made if the ascitic fluid polymorphonuclear cell count was greater than 250 mm^3, with or without positive culture, and in the absence of an intra-abdominal source of infection. Bacteriaemia was considered when clinical symptoms and signs of infection were present and confirmed by the microbiological demonstration of the causative organism from the blood culture in the absence of site-specific infection.

Ethical considerations

The study protocol was approved by the Ethical and Science Committee of the University of Debrecen. Each patient was informed of the nature of the study and signed an informed consent form.

Determination of the MBL level

We used a double-antibody sandwich ELISA system adopted from Minchinton et al. [10] to determine MBL levels. Briefly, microtiter plates (flat bottom, high binding capacity, Greiner Bio-One, Mosonmagyarovar, Hungary) were coated for overnight incubation at 4 °C with 1 μg/ml monoclonal mouse anti-human MBL antibody (clone 131-1; BioPorto Diagnostics A/S, Gentofte, Denmark) in Tris-buffered saline (TBS). Three dilutions of the sera (1/5, 1/25, 1/125) were then incubated for 90 min at 37 °C in a wet chamber together with a serial dilution of a MBL standard (BioPorto Diagnostics A/S). A vial of standard solution was assigned an MBL content of 1000 AU and we accepted that it corresponded to 3200 ng/ml oligomerized MBL, as declared by the manufacturer. After washing three times, biotinylated Mab 131–1, diluted 1:8000 in TBS with 0.05% Tween-20 and 0.25 μM EDTA (TBS-T-EDTA), at a pH 7.5 was added for 90 min at 37 °C in a wet chamber and followed by another washing step. Avidin–biotinylated-peroxidase-conjugate (Vectastain, Vector Laboratories Inc., Burlingame, CA) at 1:1000 dilution was added and incubated for 30 min at room temperature in a wet chamber. Color was developed with tetramethyl-benzidine dihydrochloride (TMB, Sigma–Aldrich, Schnelldorf, Germany), stopped with 2 M H_2SO_4, and read immediately at 450 nm in an Infinite 200 plate reader (Tecan Austria GmbH, Salzburg, Austria). The calculation of results was performed using the Magellan software program with Marquardt curve fitting. Between runs, coefficients of variation (CV) were 11.3%, 12.3%, and 11.6% at 1:5, 1:25