

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

Bacterial infections in cirrhosis: diagnosis and prognosis

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The Examination takes place at the Library of Division of Clinical Laboratory Science, Department of Laboratory Medicine, University of Debrecen, May 19, 2017, 11:00 AM.

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INTRODUCTION

In Hungary chronic alcohol consumption, viral infections and obesity are the most common causes of cirrhosis. Autoimmune diseases of the liver, inherited metabolic diseases or other rare conditions are less frequent. Nowadays liver cirrhosis is the 6th leading cause of death in Hungary. The advances in the treatment of viral hepatitis led to a reduction of liver-related deaths however, the rising number of people affected by alcohol misuse and the epidemic of obesity may turn around this tendency. Chronic liver disease is often asymptomatic, therefore patients are commonly diagnosed already with decompensated cirrhosis with one or more complications (upper gastrointestinal hemorrhage, hepatic encephalopathy, development of ascites, jaundice and hepatocellular carcinoma).

Accordingly, chronic liver disease is frequently not stable, but after a period of quiescent compensated stage, progression to decompensated cirrhosis to end-stage liver disease occurs, that eventually results in death in the absence of liver transplantation. During the course of natural progression, a group of patients experience an acute deterioration requiring hospital admission that is referred to as acute decompensation. A proportion of patients will develop one or more (extra)hepatic organ-failure(s), characterized by high mortality rate (50-90%). This clinical scenario was recently defined as acute on chronic liver failure (ACLF) in the medical literature. Identification of the precipitating event such as variceal bleeding, active alcohol consumption is often possible, but the most frequent acute insults are bacterial infections. Bacterial infections are therefore of paramount importance in patients with cirrhosis, moreover once present they lead to a four-fold increase in one-year mortality, regardless of the severity of hepatic fibrosis.

Early diagnosis of bacterial infections is essential to prevent complications of liver failure and death in this patient group. However, in the clinical practice their accurate identification is challenging from both the clinical and the laboratory part. In cirrhosis, usual clinical presentations lack up to 50% of the bacterial infections and are replaced by non-specific complaints or just revealed by organ dysfunctions. Due to some disease specific characteristics, there is an evident lack of sensitivity and specificity of the conventional laboratory and clinical parameters for the definition of systemic inflammatory response (SIRS), that makes difficult to diagnose sepsis.

Currently C-reactive protein (CRP) and procalcitonin (PCT) are broadly used in

the clinical practice to aid the early diagnosis of bacterial infections. In cirrhosis, these conventional markers, however, perform somewhat differently compared with non-cirrhotic patient populations for various reasons. For the first, if the main source of the molecule is the liver, like in the case of CRP, synthesis of the molecule is affected by liver failure and its severity. As a result, the diagnostic accuracy of liver synthesised acute phase proteins (APPs) decreases in advanced stage of cirrhosis. Moreover, peak levels can be misleading and do not indicate the severity of the infection adequately, since the more severe the underlying liver dysfunction, the lower the CRP response to bacteraemia is. Secondly, elimination of certain molecules can be affected by renal failure and renal replacement therapy. Acute kidney injury (AKI) is frequent in patients with cirrhosis, especially in bacterial infections. While CRP has a high molecular weight (115-kDa) and its renal clearance is negligible, PCT is small (13 kDa) and renal elimination is thought to be one of the major pathways for its elimination. Thirdly, inflammatory state sustained by bacterial translocation (BT) without overt infection is sufficient by itself, to elevate inflammatory markers.

Accordingly, data are not homogeneous about the optimal cut-off for either of CRP and PCT to differentiate patients with infection from those without. Probably using a single threshold is not appropriate. Additional biomarkers are highly needed to optimize the rule in and rule out processes necessary for the diagnosis and for the severity assessment of bacterial infections in cirrhosis.

Review of the literature

Presepsin (soluble CD14 subtype, sCD14-ST) is a 13-kDa cleavage product of CD14 receptor and can be perceived as a witness of activated monocyte-macrophage in response to pathogens. Several recent clinical studies have shown, that presepsin is a specific and sensitive novel marker for the diagnosis of sepsis, for evaluating the severity of sepsis and for predicting the outcome. Beyond sepsis, presepsin is worthy of studying in those clinical settings, where systemic infections are frequently associated with severe diseases course such in cirrhosis (acute decompensation [AD], organ failure). Contributive role of presepsin for the diagnosis and prognosis of cirrhosis associated bacterial infection has not been assessed extensively so far.

Previously our research group has shown that CRP and other APPs predict the development of bacterial infections in cirrhosis. These biomarkers however, represent the

proinflammatory process that is prominent in these patients. It is known that compensatory anti-inflammatory response (CARS) parallels proinflammation and is responsible for the down-regulation of inflammation and the elimination of tissue debris. Overwhelmed CARS thus, is leading to impaired elimination of bacterial infections. Therefore it is reasonable to assume that the extent of CARS has a role in the prognosis of AD and ACLF.

Resident macrophages of the liver, Kupffer cells, are important cellular components of the innate immune system, and have a central role in the orchestration of the pro- and antiinflammatory response. During inflammation, the haemoglobin-haptoglobin scavenger receptor, CD163, is cleaved from the surface of macrophages. This soluble form of CD163 (sCD163) is thereafter detectable in the systemic circulation and regarded as a marker of macrophage activation. Binding and internalization of the haemoglobin-haptoglobin complex is a well-described function of this receptor, but several other functions have also been attributed to CD163. CD163 is expressed on M2-type (pro-resolution, anti-inflammatory) liver macrophages in the presence of local microenvironmental anti-inflammatory signals such as interleukin (IL)-10. Accordingly, high serum level of sCD163 has been considered as a representative marker of the anti-inflammatory response. Recently, monocytes and macrophages of anti-inflammatory properties were highlighted in the pathogenesis of AD and development of organ failure in cirrhosis.

Increased serum sCD163 level were found in non-cirrhotic patients during bacterial infection and even higher levels in sepsis. An association between increased sCD163 level and reduced survival has also been reported. It was also demonstrated that in cirrhosis the sources of elevated sCD163 levels were the macrophages of the liver. However, no data are available regarding the significance of macrophage activation represented by sCD163 serum level during bacterial infection and/or other AD episodes of cirrhosis.

AIMS OF THE STUDY

We examined a large prospective cohort of cirrhotic patients, and aimed to examine:

1. whether presepsin as an acute phase protein:
 - 1.1 can be used in the diagnosis of bacterial infections, compared to routinely used APPs such as CRP or PCT.
 - 1.2 is devoid of the limitations of classic APPs related to cirrhosis.
 - 1.3 is able to provide prognostic information during bacterial infections in cirrhosis.
2. whether soluble CD163 as an anti-inflammatory biomarker
 - 2.1 is able to provide prognostic information during acute decompensation in cirrhosis.

PATIENTS AND METHODS

Patient Population

We performed a cohort study among adult patients with an established diagnosis of cirrhosis of different aetiologies at the Division of Gastroenterology Department of Internal Medicine, Clinical Center, University of Debrecen. Patients were included consecutively from the outpatient clinic during regular or extraordinary follow-up visits and from the inpatient ward owing to hospitalization with an AD episode.

For the present study, clinical data and biologic samples (sera and plasma) of patients recruited between May 1, 2006 and April 30, 2011 were used. Diagnosis of cirrhosis was based on clinical, biochemical, imaging and when available, histological data. Blood samples, routine laboratory data and detailed clinical phenotype were captured at inclusion. Detailed clinical data were recorded at enrolment. Sera and plasma of patients were collected at time of routine laboratory examinations. Clinical data were determined by in-depth review of the patients' medical records using a structured interview. At inclusion, severity of liver failure was assessed by liver-oriented scores (Child-Pugh and model for end-stage liver disease [MELD]). If present, the type of the AD episode was established. Acute decompensation was defined by one or any combination of the following events: development of large ascites, hepatic encephalopathy, gastrointestinal haemorrhage and bacterial infection. Presence of systemic bacterial infection was carefully established in all patients with AD episode by compatible clinical symptoms, laboratory data, results of urine analysis, imaging findings and the result of a diagnostic tap if ascites was present.

The following infections were diagnosed: (1) spontaneous bacterial peritonitis: neutrophil cell count $>250/\text{mm}^3$ 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 $>10/\text{mm}^3$) 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, orocavitary, intestinal tract infection, osteomyelitis, endocarditis; (5) bacterial infection with unknown origin: positive blood culture in the absence of site-specific infection. Presence and grade of organ system failure(s) [OF] were determined retrospectively based on the available clinical and laboratory data after accessibility of CLIF-C Organ Failure Score in 2013.

The control group consisted of 150 age- and gender-matched healthy blood donors (male/female: 72/78, age: 51.5 ± 16.9 years).

Outcome

Short-term mortality was defined as death that occurred in the first 28 days, since mortality after 28 days is often attributed to a subsequent readmission or other precipitating clinical events.

A presepsin, a sCD163 és egyéb laborparaméterek mérése

Routine laboratory data, such as liver biochemistry, renal function, blood count and serum CRP and PCT levels were determined directly at the Department of Laboratory Medicine. For serologic investigations sera and plasma of patients were immediately centrifuged at 3000g for 10 minutes, and stored at -70 °C until further use.

Presepsin levels were measured by means of a PATHFAST® presepsin analyzer (Mitsubishi Chemical Medience Corporation, Tokyo, Japan) which is based on chemiluminescent enzyme immunoassay, with a detection limit of 20 pg/mL.

Serum level of sCD163 was determined by ELISA, according to the manufacturer's instructions (IQProducts, Groningen, Neatherlands), the limit of detection was 0.23 ng/ml. Samples were measured in duplicates on the same plate, and the mean values were used. Between runs, coefficients of variation were 8%.

Serological assays were performed at the Department of Laboratory Medicine in a blinded fashion without prior knowledge of the patient's clinical information.

Ethical permission

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 this study and signed an informed consent form.

Statistical analysis

Variables were tested for normality using Shapiro Wilk's W test. Variables were summarized as means (standard deviation), medians (IQR, lowest 25% to highest 25%),

or as number (percentage). Variables were compared with Fisher's exact, Chi-square, Mann–Whitney U-, or Kruskal–Wallis H-test as appropriate. Paired samples were analyzed by Wilcoxon signed-rank test. The Spearman's nonparametric rank correlation test was used to determine correlations. Ability of different variables to discriminate between groups of patients were assessed by receiver operating characteristics curve (ROC) analysis. Youden index was chosen, to estimate the best discriminate threshold between groups. Sensitivity, specificity, positive and negative predictive values were calculated. ROC curves were compared with the method of DeLong. Kaplan–Meier curves were used to estimate survival, time to event failures were compared with the log-rank test. The association between categorical and continuous variables and the time of death during acute decompensation was assessed with Cox-regression analysis and the backward elimination procedure. Binary logistic regression was used when short-term mortality was considered as a dichotomous variable. Variables were logarithmically transformed for regression analyses. Associations are given as hazard ratio (HR) or odds ratio (OR) with 95% confidence interval (CI). For statistical analyses and graphical presentations, the SPSS 22.0 (SPSS, Chicago, IL, USA) and GRAPHPAD PRISM 6 (San Diego, CA, USA) programmes were used (with the supervision of a qualified statistician, Zsolt Karanyi). A two-sided probability value of <0.05 was considered to be statistically significant.

RESULTS

Patient characteristics – Presepsin

Two hundred and sixteen patients with cirrhosis were enrolled in this cohort. There were 118 men with a mean age of 57.6 ± 10.3 years. The median Child-Pugh and MELD score were 8 (IQR: 6-10) and 14 (IQR: 10-17), respectively. Etiology of cirrhosis was alcoholic in 159 (73.6%), and viral in 46 (21.3%) patients, seven cases were of autoimmune origin and 4 cases with other rare chronic liver diseases. 117 patients (54.2%) had extrahepatic co-morbidities. Renal impairment was present in 33 (15.3%) patients based on creatinine cut-off values $\geq 133 \mu\text{mol/L}$. 15 patients (7%) had hepatocellular carcinoma. Acute decompensation of the disease warranting hospital admission occurred in 101 patients (46.8%) of whom 27 (26.7%) had at least one organ failure.

Documented bacterial infection was present in 75 (34.7%) patients of whom 9 (12.0%) suffered from multifocal episode. The most frequent bacterial infections were: (1) urinary tract infection ($n=25$), (2) spontaneous bacterial peritonitis ($n=20$) and pneumonia ($n=18$). The infected and the non-infected patient groups did not differ in gender, age, and presence of comorbidities. Organ failure was more common in patients with bacterial infections compared to those without (37.5% vs. 8.1%, $p=0.001$), and was mainly due to renal failure (29.3% vs. 7.8%, $p<0.001$).

Association between presepsin levels and bacterial infections

Presepsin values ranged from 142 to 5950 pg/mL (median [IQR], 576 pg/mL [376-972]) and were significantly higher in patients with infection as compared to those without (1002 pg/mL [575-2149] vs 477 [332-680] pg/mL, $p<0.001$). This association was also confirmed in the different disease severity subgroups according to Child-Pugh stage or the presence of ascites. In the subgroup of patients with renal failure, presepsin levels were also different numerically between infected and non-infected patient groups however it did not reach statistical significance (2162 [1311-2813] vs. 1011 [667-228], $p=0.082$).

Further evaluating non-infected patients, a significant increase was observed in presepsin levels in case of more advanced disease stage and also in the presence of renal failure ($p<0.001$, for all).

Presepsin level positively correlated with classic markers of bacterial infections (Rho_{CRP} : 0.63, Rho_{PCT} : 0.53, $Rho_{leukocyte}$: 0.27), and liver oriented scores (Rho_{cPs} : 0.42, Rho_{MELD} : 0.45).

Presepsin level was not different according to the location or Gram specificity of the infection (Gram-negative: 1240 [871-1884] vs. Gram-positive: 852 [661-2467], $p=0.76$). 24 infections (32%) were complicated by at least one OF. Presepsin level was significantly higher in patients with OF as compared to those without (2358 pg/mL [1398-3666] vs. 710 pg/mL [533-1277], $p<0.001$).

Accuracy of presepsin level in the diagnosis of bacterial infections compared to classic acute phase protein

The diagnostic accuracy of presepsin for identifying patients with infection was established by ROC analysis and compared to CRP and PCT. Overall Presepsin was a similar to PCT in predicting bacterial infections (AUC-ROC, 95%CI: 0.79 [0.73-0.84] vs. PCT 0.77 [0.71-0.83], $p=0.668$), but somewhat lower than CRP (0.86 [0.80-0.90], $p=0.057$). The best cut-off value that discriminated patients with infections was 844pg/mL. At this cutoff the sensitivity, specificity was 60% and 84.4%, respectively. After determining the best cut-off values of the other APPs (CRP: 10.8mg/L, PCT: 0.39 μ mol/L) we examined whether using these markers in combination can improve the laboratory diagnosis of bacterial infections. Combination of CRP with presepsin increased the sensitivity by 9 % (Sensitivity $_{CRP}$ vs. $_{CRP + Presepsin}$: 78.7% vs. 87.5%). On the contrary, the diagnostic accuracy of presepsin [AUCROC 95%CI: 0.85 (0.74-0.92)] for identifying patients with infection complicated by OF was similar to PCT (0.85 [0.74-0.92]) and clearly superior to CRP (0.66 [0.54-0.77], $p=0.994$ for presepsin vs PCT, and $p<0.01$ for presepsin vs. CRP)

Association of presepsin level with short-term mortality during infectious episodes

Of the 75 patients with bacterial infection, 20 patients (27.4%) died within the first 28 days. Plasma presepsin levels at admission were significantly higher in non-survivors than in survivors [2323 (1172-3688) vs. 852 (549-1451) pg/mL, $p<0.001$]. Discriminative ability (AUC-ROC) of presepsin was 0.76 with the best cut-off value of 1277 pg/mL. 28-day mortality rate above this threshold was 46.9% versus 11.6% ($p<0.001$) that was observed in patients with presepsin below this level. In the univariate logistic regression analysis,

increased presepsin level was found to be a risk factor of short-term mortality during bacterial infection [OR = 3.59 (95%CI: 1.65-7.84), P = 0.001] similarly to CRP and PCT. Presepsin level however lost its significance after adjusting for MELD score and leukocyte count (1.61 [0.65-3.97], p=0.303), in multivariate binary logistic regression analysis.

Patient characteristics – sCD163

378 patients were included (193 stable outpatients and 185 patients with acute decompensation). Baseline characteristics of patients were similar to those included in the presepsin study. In the total patient population serum values of sCD163 ranged from 279 to 28818 ng/ml and were significantly higher compared to healthy subjects [median (IQR), 3852 ng/ml (2265–6542) vs. 1104 (863–1438), p<0.001]. sCD163 level increased gradually according to disease severity, as rated by the Child-Pugh stage [A: median (IQR): 2984 (1839–4999), B: 3838 (2392–6432) and C: 5917 (3235–8266) ng/ml, p<0.001 for all].

Significant correlation was found between sCD163 level and laboratory markers of inflammation, impaired renal and liver function. The most prominent were GPT (Rho: 0.35), MELD score (Rho: 0.30) and Child-Pugh score (Rho: 0.32).

sCD163 level in patients with acute decompensation episode

In a total of 185 patients with acute decompensation episode, sCD163 level was significantly higher in patients with AD episode compared to outpatients, but only in the presence of bacterial infection (median, [IQR]: outpatients: 3538 [2128-5876], AD-noninf: 3792 [2165-6389], AD-inf: 4586 [2854-8066]). sCD163 levels were significantly higher in patients with bacterial infection complicated with organ dysfunction(s), namely ACLF, compared to those without [median (IQR), 7233 ng/ml (3864–11643) vs. 3864 ng/ml (2700–7031), p=0.003].

sCD163 is associated with short-term mortality during infectious episodes

Twenty-five patients with bacterial infection (25%) died during the first 28 days of follow-up. sCD163 level at admission were significantly higher in non-survivors than in survivors (median [IQR], 7233 ng/ml [3594–10337] vs. 4045 ng/ml [2700–7355], p=0.029). MELD score (24 [20–33] vs. 16 (12–20), p<0,001), CRP level (51 mg/L [33–

93] vs. 26 mg/L [13–47] $p=0,001$) and also leucocyte count (12,1 G/L [6,3–15,4] vs. 7,2 G/L [5,1–10,1], $p=0,014$) were significantly different between the two groups. The best discriminate threshold for sCD163 level estimated by the Youden-index was 7000 ng/mL. 28-day mortality rate was 17% in patients with sCD163 concentration less than 7000ng/mL, whereas 44% in patients with sCD163 above 7000ng/mL (HR: 3.04 [1.38–6.71], $p=0.006$). After adjusting for important clinical variables (etiology, MELD score, CRP, leukocyte count) sCD163 level did remain an independent predictor of short-term mortality during bacterial infections (HR: 2.96, [1.27–6.95], $p=0.012$).

DISCUSSION

Primary aim of our study was to assess the performance of presepsin – a recently reported novel sepsis marker – in the diagnosis of cirrhosis associated bacterial infections. We also aimed to compare its capacity to routinely used APPs (CRP, PCT) in a patient cohort that represents everyday clinical practice. To the best of our knowledge, this is the first study in cirrhosis, reporting the feasibility and the usefulness of presepsin in these clinical settings. We evaluated a large cohort of patients, in that one-third of patients had mild course of infections and mainly localized to the urinary tract, while another subgroup of patients (32%) suffered in severe bacterial infections, with one or more (extra)hepatic organ failure(s). For the diagnosis of bacterial infections, the best cut-off level of presepsin was 844 pg/mL in our cirrhotic patient cohort. Diagnostic cut-off levels were different in previous studies in non-cirrhotic populations, but most reports suggest an approximate level of 400–600 pg/mL. Presepsin alone was not suitable as a screening tool to search for infections, however adding it to CRP; we found that presepsin was clinically useful, mainly for two reasons. Firstly, this combination amended efficacy of identification of the infectious episode (sensitivity increased by 9 percentage points compared to CRP alone). Secondly, compared to CRP, presepsin was more accurate in distinguishing severe infectious episode from non-severe ones, AUC-ROC values were 0.66 and 0.85, respectively. Performance of presepsin corresponds to that reported in a recent meta-analysis of *Zheng et al.* comprising a total of 8 studies and 1757 patients, the AUC of the summary ROC (SROC) was 0.82.

Secondary aim of our study was to evaluate whether presepsin is devoid of the limitations of classic APPs in cirrhosis. In a former study, we reported that the diagnostic accuracy of CRP and PCT for identifying patients with infection decreased in advanced cirrhosis. Correspondingly, presepsin behaved alike in the present study. Presepsin is not primarily synthesized in the liver, thus the major limitation of its diagnostic performance is not the decreased synthetic capacity in advanced cirrhosis. Ongoing chronic inflammatory state is a characteristic feature of cirrhosis that is potentially able to induce the synthesis of APPs in the absence of infections and inevitably limits their clinical utility in the diagnostic procedure of bacterial infections. In cirrhosis, bacterial translocation is of major importance in perpetuating chronic inflammation.

Another important, but rarely considered issue is the effect of renal function on

the levels of APPs. Acute kidney injury is a frequent (up to 50%) complication of bacterial infections in cirrhosis. Exact clearance mechanism of presepsin is unknown, but it is presumably filtered by the glomeruli, reabsorbed, and metabolized within the proximal tubular cells. *Nagata et al.* reported that presepsin levels tend to increase with decreasing glomerular filtration rate, and are markedly high in patients with chronic renal failure or receiving hemodialysis. Accordingly, we also found a significant correlation between presepsin and serum creatinine level. Furthermore, in a small subgroup of patients with renal failure, presepsin values were markedly high even in the absence of infection. These results suggest that evaluation of presepsin levels in cirrhosis warrants special consideration during AD episodes complicated by AKI, and probably a different cut-off is needed for diagnosing infections in such patients.

Third aim of our study was to assess whether presepsin is able to provide prognostic information in cirrhosis associated bacterial infections. Studies in this clinical setting only exist regarding CRP and PCT and their findings are not without controversies. Most of the studies included both stable outpatients and patients with ongoing AD episodes with or without bacterial infections. Furthermore, evaluations often were performed as a whole of these non-homogenous patient groups rendering direct comparison and a single conclusion rather difficult. In patients with increased level of PCT, CRP and presepsin, short-term mortality was higher. Indeed, higher level of PCT, CRP and presepsin were associated with short-term mortality in our study. However, after adjusting for diseases severity and leukocyte count, this association was only preserved for PCT and not for CRP or presepsin. This finding might be explained by the fact that PCT belongs to a distinctive class of molecules, so-called "hormonkines". It is produced primarily in neuroendocrine cells of various organs and represents involvement of several instead of just one organ in the pro-inflammatory response. Procalcitonin has a cytokine-like behaviour during inflammation and infection and has various toxic effects, thus pose harm to the host. In contrast, presepsin represents activation of the monocyte-macrophage system during inflammatory process. Macrophages have a dual effect: on the one hand, they produce excessive amount of inflammatory cytokines that can cause tissue damage, on the other hand they are involved in the resolution of the inflammation and can promote tissue repair as well. The former process is driven by M1-type, or so-called proinflammatory macrophages however, the latter process is driven by M2-type macrophages in the presence of local microenvironmental anti-inflammatory signals such

as IL-10.

Soluble CD163 is shed from M2-type macrophages and has a good correlation with other soluble macrophage activation markers, such as soluble urokinase plasminogen activator receptor (suPAR), soluble mannose receptor (sMR), but does not correlate with sCD14, which is also shed from macrophages. Recently, sCD163 has emerged as a promising novel marker in cirrhosis. Enhanced formation of sCD163 is a well-known characteristic of cirrhosis and is consistently associated with advanced disease stage and portal hypertension.

Little is known however, about sCD163 levels in different types of AD. Moreover, the role of sCD163 in predicting development of complications or short-term mortality during AD or bacterial infections is still unclear. We have shown that sCD163 is increased in patients with bacterial infection. Interestingly, we found that the more severe the infection – defined by the presence of organ failure(s) – the higher the level of sCD163 was. We have shown for the first time that in cirrhosis, the 28-day mortality was associated with increased sCD163 level (>7000 ng/ml) during bacterial infection. sCD163 was an independent risk factor regardless of the disease severity and the extent of the pro-inflammatory response, represented by MELD score, CRP and leucocyte count respectively. This finding is consistent with the observations that excessive anti-inflammatory response, represented by interleukin-10, interleukin-6 or soluble tumour necrosis factor- α receptor (sTNFR) and decreased monocyte HLA-DR expression, has a significant negative impact on survival in cirrhosis, as recently reviewed by *Albillos et al.* Interestingly, various markers of the anti-inflammatory response had similar short-term mortality risk than sCD163 in our study.

The deleterious effect of the exaggerated anti-inflammatory response is attributed to the impaired response to microbial challenge. A recent study from *Bernsmeier et al.* further highlighted the role of CD163^{high} monocytes/macrophages in this process. They demonstrated that the impaired response to microbial challenge could be restored by modulating anti-inflammatory (CD163 high -MERTK positive) monocytes *in vitro*.

In everyday clinical practice, accurate and early selection of the most vulnerable patients during bacterial infection, requiring intensive care and monitoring is especially important. A representative biomarker of the altered anti-inflammatory pathway could be included in a predictive model from a pathophysiological point of view. sCD163 might be a promising candidate for this purpose. The relatively low sample size of patients with

bacterial infection in our study (n = 99) and the single centre design did not allow us to satisfy the above mentioned need. Predictive models of cirrhosis and short-term survival were developed in multicentre cohorts with much larger patient populations. Our single-point measurement approach did not allow for evaluating the potential importance of sCD163 kinetics, which is another limitation of our study.

To conclude, the present study suggests that presepsin is a promising biomarker during the diagnostic workup of bacterial infections in cirrhosis for enhancing diagnostic capacity of CRP and reflecting more accurately the severity of infections. Performance of presepsin is equal to PCT in these clinical settings. Diagnostic accuracy of presepsin, however, decreases in advanced stage of the disease or in the presence of renal failure. Furthermore, presepsin is not suitable for predicting infection-related short-term mortality in patients with cirrhosis. Distinctly, sCD163 is an independent predictor of short-term mortality in this patient group that highlights the deleterious effect of the excessive anti-inflammatory response during bacterial infection.

MAJOR SCIENTIFIC FINDINGS

- 1) We reported, for the first time, on presepsin as a potential biomarker in the diagnosis of bacterial infections in cirrhosis.
- 2) Presepsin is higher in bacterial infections, and is associated with the severity of infections. Plasma levels of presepsin are even higher in cases complicated by organ failure(s).
- 3) Presepsin enhances accuracy of CRP in the diagnosis of bacterial infections in cirrhosis.
- 4) Performance of presepsin in the identification of patients with severe infections is similar to PCT and superior to CRP.
- 5) Diagnostic accuracy of presepsin decreases in advanced cirrhosis and in the presence of renal failure.
- 6) Soluble CD163 is an independent predictor of short-term mortality in patients with cirrhosis and bacterial infection.

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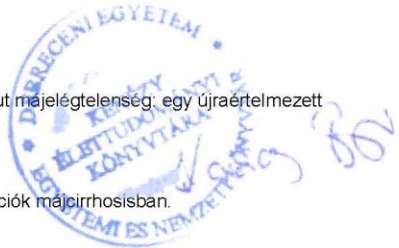
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List of publications related to the dissertation

1. **Tornai, T.**, Vitális, Z., Sipeki, N., Dinya, T., Tornai, D., Antal-Szalmás, P., Karányi, Z., Tornai, I., Papp, M.: Macrophage activation marker, soluble CD163 is an independent predictor of short-term mortality in patients with cirrhosis and bacterial infection.
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3. Kocsis, D., Papp, M., **Tornai, T.**, Tulassay, Z., Herszényi, L., Tóth, M., Juhász, M.: Intestinalis zsírsavkötő fehérje: az enterocytakárosodás markere akut és krónikus gasztroenterológiai kórképekben.
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Total IF of journals (publications related to the dissertation): 7,257

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