# Macrophage activation marker, soluble CD163 is an independent predictor of short-term mortality in patients with cirrhosis and bacterial infection

Tamas Tornai[1], Zsuzsanna Vitalis[1], Nora Sipeki[1], Tamas Dinya[2], David Tornai[3], Peter Antal-Szalmas[3], Zsolt Karanyi[4], Istvan Tornai[1], Maria Papp[1]

[1] Department of Internal Medicine, Division of Gastroenterology, [2] Institute of Surgery, [3] Department of Laboratory Medicine, [4] Department of Internal Medicine, Division of Endocrinology, Faculty of Medicine, University of Debrecen, H-4032 Debrecen, Hungary

**Corresponding author:** Maria Papp, M.D., Ph.D., 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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**List of abbreviations:** sCD163: soluble CD163, AD: acute decompensation, INF: bacterial infection, MELD: model for end-stage liver disease, ACLF: acute-on-chronic liver failure, sTNFR: soluble tumor necrosis factor-α receptor

**Conflict of interest:** none to declare

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**ABSTRACT**

**Background&Aims:** Innate immune system dysfunction is common in advanced cirrhosis, with a central role of the monocyte/macrophage system. Monocytes and macrophages express the scavenger receptor CD163 which is regulated by inflammatory mediators. Cleavage of the receptor leads to formation of soluble (s)CD163, that represents anti-inflammatory response. We aimed to study the clinical importance of sCD163 in cirrhosis.

**Methods:** Sera of 378 patients were assayed for sCD163 by ELISA (193 outpatients and 185 patients with acute decompensation[AD]). A 5-year follow-up observational study was conducted to assess the possible association between sCD163 level and poor disease outcomes.

**Results:** sCD163 level was associated with disease severity, but not with the presence of varices or prior variceal bleeding. In outpatients, sCD163 level did not predict the development of disease-specific complications or the long-term mortality. In patients with AD episode, sCD163 level was significantly higher compared to outpatients but only in the presence of bacterial infection[INF] (AD-INF:4586, AD-NON-INF:3792 and outpatients: 3538ng/mL, $p<0.015$ and $p=0.001$, respectively). sCD163 level gradually increased according to severity of infection. During bacterial infections, high sCD163 level(>7000ng/mL) was associated with increased mortality rate (42% vs. 17%, $p<0.001$) and was identified as an independent predictor of 28-day mortality (HR:2.96, 95%CI:1.27–6.95) in multivariate Cox-regression model comprising etiology, co-morbidity, MELD score and leukocyte count as covariates.

**Conclusions:** High sCD163 level is useful to identify patients with high-risk of death during an AD episode complicated by bacterial infection. This finding serves as an additional hint towards the significance of anti-inflammatory response during bacterial infection.

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**Keywords**: sCD163, macrophage activation, anti-inflammatory response, cirrhosis, bacterial infection,

**Key Points**

- The present 5-year follow-up cohort study comprehensively evaluated the significance of macrophage activation marker, soluble (s)CD163 in the prediction of poor disease outcomes in cirrhosis.
- Previous findings that high serum level of sCD163 was an independent predictor of variceal bleeding and long-term mortality has not been confirmed. sCD163 level did not predict development of other long-term complications (ascites formation, hepatic encephalopathy, and bacterial infections).
- During bacterial infection, a high sCD163 level was a risk factor of short-term mortality independently from disease severity and the extent of pro-inflammatory response.
- These results highlight the deleterious effect of the excessive anti-inflammatory response during bacterial infection.

## Introduction

Leading causes of hospitalization in patients with cirrhosis include hepatic encephalopathy, upper gastrointestinal bleeding, bacterial infections and rapid accumulation of ascites; collectively referred to as acute decompensation (AD) [1]. During AD episodes a proportion of patients newly develop liver and/or extrahepatic organ failure(s), a distinct clinical entity defined as acute-on-chronic liver failure (ACLF) [1]. ACLF has recently gained great interest for several reasons. The syndrome is

associated with high short-term mortality, but precipitating factors and the exact pathogenetic processes have not yet been completely identified [2]. Bacterial infections clearly have an important role [3]. Various clinical factors have been established as predictors of short-term mortality in patients with bacterial infection [4]. Biomarkers identifying relevant pathophysiological pathways in patients with infection potentially may add value to understanding and managing this condition [5].

Monocytes and macrophages are important cellular components of the innate immune system. Resident macrophages of the liver, Kupffer cells, have a central role in the orchestration of pro- and anti-inflammatory processes and have been studied extensively in chronic liver diseases [6]. During inflammation the haemoglobin-haptoglobin scavenger receptor, CD163 is cleaved from the surface of macrophages. This soluble form (sCD163) is thereafter detectable in the systemic circulation and regarded as a marker of macrophage activation [7,8]. 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 [8]. 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 [9,10]. Accordingly, high serum level of sCD163 has been considered as a representative marker of the anti-inflammatory response in a number of clinical studies [11–14] Recently, monocytes and macrophages of anti-inflammatory properties were highlighted in the pathogenesis of AD and development of organ failure in cirrhosis [15,16].

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 [11,12,17,18]. In cirrhosis, increased sCD163 level was reported in patients with significant portal hypertension or advanced disease stage [19–21]. It was also demonstrated that the sources of sCD163

were the macrophages of the liver [20]. Moreover, in a study of *Waidmann et al.* [22], sCD163 level was found to be a prognostic marker of overall survival and the development of variceal bleeding. Development of other long-term complications (i.e. hepatic encephalopathy, ascites and bacterial infections) of cirrhosis in relation to sCD163 level however, has not been yet evaluated. In addition 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.

The aim of the present study was to investigate the clinical importance of sCD163 in a large referral patient cohort with cirrhosis. The primary aim was to assess the association between serum sCD163 level and the disease specific characteristics (disease severity, portal hypertension or presence and type of the acute decompensation event). Secondary aim was to evaluate whether sCD163 level is able to predict poor disease outcomes in cirrhosis. (1) In stable outpatients we evaluated the advent of decompensation events (ascites formation, hepatic encephalopathy, variceal bleeding, and/or bacterial infections) and long-term mortality. (2) In patients with acute decompensation we evaluated short-term mortality with careful distinction of 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). This cohort was reported in previous serological studies of our group [23,24]. Between May 1, 2006 and December 31, 2010, patients were included

consecutively from the outpatient clinic during regular or extraordinary follow-up visits and also from the inpatient ward due to hospitalization with an AD episode. The exclusion criteria were (1) if the patient or his/her legal surrogate declined to participate in the study and did not sign the informed consent or (2) if the patient was sent just for specialist consultation, and followed-up regularly elsewhere. For present study serum samples of 378 patients (193 outpatients and 185 hospitalized subjects due to an AD episode) were available.

Clinical characteristics of the patients at inclusion are presented in **Table 1**. 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. 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 esophageal varices, history of previous AD episode(s), presence of hepatocellular carcinoma, extrahepatic co-morbidities (myocardial infarction, congestive heart failure, peripheral arterial disease, cerebrovascular disease, chronic pulmonary disease, chronic renal failure, diabetes mellitus, extra-hepatic malignant disease) and cirrhosis-related medication were retrospectively analyzed for the period prior to the observational follow-up study. Indications for non-selective beta-blockers either in primary or secondary prophylaxis of variceal bleeding were considered on the recommendation of current guidelines [25]. Indications for proton pump inhibitors were gastroesophageal reflux disease, erosive gastritis, peptic ulcer disease or treatment for *Helicobater pylori* infection [26]. At enrolment, disease severity assessed by liver-oriented scores (Child-Pugh and MELD) were determined. 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 hemorrhage and

bacterial infection. Development of large ascites was defined by grade II/III ascites, according to the International Club of Ascites [27], patients with refractory ascites were not included. Acute hepatic encephalopathy was defined by acute worsening of mental status in a previously conscious patient, without the evidence of acute neurological disease [28]. Acute gastrointestinal bleeding was diagnosed by esophago-gastro-duodenoscopy, and was attributed to acute variceal bleeding according to conventional criteria [29]. Presence of systemic bacterial infection was carefully established by compatible clinical symptoms, laboratory data (leucocyte count, high-sensitivity C-reactive protein [CRP] and procalcitonin [PCT], 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 in all patients with AD episode. 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) [23] were considered to support the diagnosis of infection. Based on specific clinical symptoms and findings the following infections were diagnosed. (1) Spontaneous bacterial peritonitis (SBP): 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.

Further characterization of bacterial infections was done on the basis of conventional criteria [30–33]. Additionally, during AD events presence of ACLF and its grade was also assessed, although only retrospectively after the new definition of ACLF became available in September, 2013 [1].

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

**Phenotypical characterization of patients during follow-up**

Patients were enrolled into an observational follow-up study, where the attending gastroenterologist registered laboratory data, imaging and endoscopic findings, disease severity, medical treatment, date and type of disease specific complications during regular and extraordinary outpatient follow-up visits and inpatient stays. In Hungary, a regular outpatient follow-up visit is usually scheduled for every 3 months at a specialized gastroenterology center for patients with decompensated cirrhosis (a follow-up between 1-3 months may be scheduled if dictated by disease severity or presence of certain disease specific complications) and for up to 6 months for patients with cirrhosis but without prior episode of AD. The follow-up period lasted 5 years, or death/loss of follow-up. The median follow up was 661 days (IQR: 104-1563).

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 complications, (i.e. occurrence of ascites formation, variceal bleeding, hepatic encephalopathy, clinically significant bacterial infection or death were defined as adverse outcomes.

**Serological analysis**

Blood samples were obtained at enrolment from each patient and were frozen at -70°C until testing, 2013 May. Very high stability of the molecule over time and different conditions were reported previously. sCD163 was resistant to repeated freezing and thawing. [8,34]. Serum level of sCD163 were determined by a solid-phase enzyme-linked immunoassay, according to the manufacturer's instructions (IQProducts, Groningen, Netherlands), in a blinded fashion. Samples were measured in duplicates on the same plate, and the mean values was used. Between runs, coefficients of variation (CV) were 8%. The limit of detection was 0.23 ng/mL. sCD14 evaluation was performed previously [23], using ELISA (Quantikine, R&D Systems, Minneapolis, MN). Serological assays were performed at the Department of Laboratory Medicine in a blinded fashion by a qualified analyst (E.T.) without prior knowledge of the patient's clinical information.

**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 $\chi^2$ 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. Ability of different variables to discriminate between survivors and non-survivors in patients with bacterial infection were assessed by receiver operating characteristics curve (ROC) analysis plotting sensitivity vs. 1-specificity. Area under the curve (AUROC) and corresponding 95 % confidence intervals (CI) were calculated. Youden index was chosen, calculated as the maximum (sensitivity+specificity-1) value, to estimate the best discriminate threshold. Sensitivities, specificities, positive predictive values (PPV) and negative predictive values (NPV) were calculated at the best discriminate threshold of the variables. ROC curves were compared with the method of DeLong et al. in Medcalc. Kaplan–Meier survival curves were plotted to estimate the cumulative probability of 28-day survival during AD episode in patients with or without bacterial infections. Differences in observed probabilities were assessed by the log-rank test. The association between categorical clinical variables or sCD163 serum level and adverse disease outcomes during follow-up were 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]. We aimed to help the interpretation of the results of the multivariate Cox regression table in patients with bacterial infection, in which HRs of categorical and continuous variables are presented. For this data visualization purpose we applied the method developed by *Liu et al*. [35]. We used PROC PHREG in SAS (version 9.1.3, SAS Institute, Carry, NC), with BASELINE statement to output estimated survival function at day 28 for each combination of the explanatory variable values present in the dataset and selected for visualisation, and then was plotted against the selected continuous variable (leukocyte count). 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 values of sCD163 ranged from 279 to 28818 ng/mL in the total patient population and was significantly higher compared to healthy subjects (median [IQR], 3852 ng/mL [2265-6542] vs. 1104 ng/mL [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). Further evaluating this association in outpatients and patients with AD separately yielded the same result (data not shown).

Significant correlation was found between sCD163 level and laboratory markers of inflammation, impaired renal and liver function and accordingly with liver-oriented scores (Child-Pugh and MELD), and also with markers of portal hypertension. Non-parametric correlations are summarized in **Supplementary Table 1**.

### sCD163 level in outpatients

In a total of 193 outpatient subjects, sCD163 level was not associated with the presence of portal hypertension characterized by the presence of ascites (**Fig. 1A**), prior episodes of variceal bleeding or presence of esophageal varices (**Fig. 1B**).

We then analyzed the association between sCD163 level and poor disease outcomes (advent of AD events and liver-related death).

Seventy-seven patients (39.9%) developed some type of AD episode. Forty-four (57%) of them had more than one episode during the long-term follow-up (median [IQR], 939 [323-1825] days). The distribution of different AD episodes is shown in **Table 1**. The median time to first AD episodes was 511 days [109-840]. Serum sCD163 level

did not predict the development of ascites (HR: 0.90, 95%CI: 0.46-1.75) or the advent of episodes of variceal bleeding (HR: 0.92, 95%CI: 0.46-1.82), hepatic encephalopathy (HR: 1.47, 95%CI: 0.86-2.51) and clinically significant bacterial infection (HR: 1.32, 95%CI: 0.88-1.96) in univariate Cox models. The sensitivity analysis performed in patients with no previous decompensation event, after excluding patients with a prior AD episode (n=88) yielded the same result regarding the development of disease specific complications (data not shown).

Fifty patients (25.9%) died of liver-related complications. The median time to death was 542 days (IQR: 178-756). Univariate survival analysis, however, demonstrated a significantly worse survival in patients with increased sCD163 level (HR: 1.72, 95%CI: 1.15-2.57, p=0.008) (**Table 2A**). Further evaluation of this association in a multivariate Cox-regression model comprising age, gender and clinical variables showed only clinical factors (advanced disease according to Child–Pugh stage and presence of co-morbidity, p<0.001 and p<0.01, respectively), but not sCD163 level (HR: 1.38, 95%CI: 0.91-2.08, p=0.127) were independent predictors of long-term survival in outpatients (**Table 2B**).

**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 (**Fig. 2A**). We were also able to confirm this association intra-individually. In a subgroup of stable outpatients (n=33), during a subsequent AD episode with an ongoing bacterial infection, sCD163 level showed a significant increase (median [IQR], 3210 [2024-7364] vs. 5119 ng/mL [2940-9761], p=0.038). Median time between sample procurements was 220 days [48–999]. As a control, outpatients (n=59) having a subsequent sample from a later outpatient visit with comparable time-lag

(median, IQR: 261 [113-1046] days) were also assessed, however, sCD163 level did not show significant change (3200 [2304-5517] vs. 3857 ng/ml [2477-5669], p=0.72).

Accordingly, we further evaluated the 99 infectious cases. Distribution of the different clinically significant infections was the following: 22,2% spontaneous bacterial peritonitis, 15.2% pneumonia, 22.2% urinary tract infection, 7.1% miscellaneous, 15.2% unidentified and 18.2% of the cases were multifocal. Bacteria were Gram-negative in 52.6% and Gram-positive in 47.4% of culture-positive cases. sCD163 level was not different according to either the location or Gram specificity of the infection. Moreover, patients with multifocal infections had similar sCD163 level to those with unifocal ones (data not shown). Severity of organ failure was significantly higher in infectious AD cases as compared to non-infectious AD cases based on both the results of the MELD score (median [IQR]: 17 [14-22] vs. 14 [11-20], p=0.005) and the proportion of patients with ACLF (29.3% vs.15.1%, p=0.022). Nonetheless, sCD163 level was associated with the severity of the infection. sCD163 levels were significantly higher in patients 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 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 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]), CRP level (51 mg/l [33-93] vs. 26 mg/l [13-47]) and also leukocyte count (12.1 G/l [6.3-15.4] vs. 7.2 G/l [5.1-10.1]) were significantly different between the two groups (p<0.001, p=0.001, p=0.014, respectively).

Prognostic accuracy of sCD163 level for predicting short-term mortality in

patients with bacterial infection was established by ROC analysis and compared to MELD score and CRP. sCD163 was similar predictor of short-term mortality compared to CRP (AUROC [95% CI]: 0.65 [0.54-0.74]) vs. 0.73 [0.63-0.81], p=0.31) but inferior to MELD score (0.83 [0.74-0.90], p<0.01).

The best discriminate threshold for sCD163 level estimated by the Youden-index was 7000 ng/mL, with sensitivity, specificity, PPV, NPV of 56.5%, 72.9%, 41.2%, and 83.1%, respectively. The optimum threshold for CRP and MELD was >30 mg/l and a score of >21, respectively. Their performance belonging to the cut-off values are shown in **Supplementary Table 2.**

In the univariate Cox-regression analysis, patients with high sCD163 level (>7000 ng/mL) and bacterial infection had significantly higher risk of short-term mortality when compared to those with low sCD163 level (HR: 3.04, [95%CI: 1.38-6.71], p=0.006). Using this derived cut-off in patients without bacterial infection, however, elevated level of sCD163 was not associated with short-term mortality. Kaplan-Meier survival curves for subgroups of AD patients with or without bacterial infection is demonstrated in Figure 3. Of the clinical factors, disease etiology and presence of co-morbidities but not the patients' age, gender or the presence of ascites and prior AD episode within 6 months were significantly associated with short-term mortality. Furthermore, disease severity depicted by MELD score and increased level of markers related to pro-inflammatory response (CRP and leukocyte count) were also significantly associated with short-term mortality in patients with bacterial infections (**Table 3**).

Multivariate Cox regression analysis indicated that sCD163 level did remain an independent predictor of short-term mortality during bacterial infection (HR: 2.96, [95%CI: 1.27-6.95], p=0.012), next to presence of co-morbidities, disease etiology, high MELD score (> 21) and leukocyte count, whereas CRP lost its significance (**Table 3**). To visualize the impact of sCD163 level on short-term mortality, we plotted the

estimated event rates according to leukocyte count as a continuous variable in the four different subgroups of patients (**Fig. 4**)**.** In the case of a high level of sCD163, the 28-day mortality rate is increased both in groups with high or low MELD score at any given value of leukocyte count.

## Discussion

The soluble form of CD163 scavenger receptor has long been known as a surrogate parameter of macrophage activation in various diseases [8], and has a good correlation with other soluble macrophage activation markers, such as soluble urokinase plasminogen activator receptor (suPAR), soluble mannose receptor [12,36,37], but does not correlate with sCD14 [38], that is also shed from macrophages. In the present study however sCD163 had a significant but inverse correlation with sCD14.

Recently sCD163 has emerged as a promising novel marker in cirrhosis [22]. Enhanced formation of sCD163 is a well-known characteristic of cirrhosis and is consistently associated with advanced disease stage in the few currently available clinical studies [19,20]. Nonetheless, changes in the serum level of sCD163 during various types of AD episodes are less known and there are still many questions regarding its predictive role in the development of disease specific complications during the disease course. This is the rationale for the present study in which we comprehensively assessed the utility of serum sCD163 level in a large prospective cirrhotic patient cohort with a careful assessment of acute deterioration.

First, we evaluated the association between sCD163 level and the disease specific characteristics of the cirrhotic patients. We were able to confirm previous findings that in cirrhosis, sCD163 level does increase up to the three-fold higher than the value observed in control subjects and displays a gradual increase according to disease severity [19,20]. Median sCD163 values in our cohort corresponded to those

reported previously.

Association of sCD163 level to clinically significant portal hypertension was extensively assessed in cirrhosis previously, but yielded somewhat conflicting results. sCD163 level was positively correlated to portal hypertension assessed by invasive hemodynamic measurement [20]. Interestingly, alleviation of portal pressure by TIPS insertion was not followed by a significant drop in sCD163 level. A later study by the same Danish group [19], revealed that significant correlation between sCD163 level and portal hypertension was only present in early, but not in advanced disease stages. Contrary to invasive hemodynamic studies, sCD163 level did not differ according to presence or absence of esophageal varices or in acute bleeding [22]. Correspondingly, we also found that the sCD163 level was not associated with the presence of varices, prior or recent variceal bleeding episodes.

In the present study, sCD163 level was neither associated with poor disease outcomes in outpatients, nor with the development of disease-specific complications or the overall survival during follow-up. In cirrhosis, one single prospective study [22] assessed the predictive value of sCD163 level during the disease course, and reported that increased sCD163 level was an independent predictor of variceal bleeding and long-term mortality. That large consecutive single center cohort (n=244) comprised both outpatients and subjects hospitalized due to acute complications of cirrhosis. Thirty percent of their patients had a concomitant bacterial infection. Regarding clinical characteristics, a comparable patient population was included in our study, but a different approach was applied for the evaluation. Outpatients and patients with ongoing AD episodes were not included in one single study group, as in the research of *Waidmann et al.*, but were considered as two distinct patient populations for several reasons. On the one hand, infectious episodes represent particularly important causes of progression of liver failure and development of liver-related complications in patients

with cirrhosis [5]. Moreover, regardless of the severity of hepatic insufficiency, development of bacterial infection significantly increases mortality rate. In-hospital mortality of cirrhotic patients with infection is more than twice that of patients without infection [39]. On the other hand, sCD163 level increases during bacterial infections both in non-cirrhotic [11,12] and cirrhotic [22] patient populations. *Waidmann et al.* demonstrated significantly higher level of sCD163 in cirrhotic patients (n=79) with bacterial infections. However, in their paper it was also stated that the sCD163 level remained constant for several weeks and was not substantially affected by acute bacterial infections during sequential examination of a small group of patients (n=7). As for our cohort, we have shown that sCD163 is increased in patients with bacterial infection. We were able to confirm this association intra-individual as well as in a larger group of patients (n=33). Furthermore we have also shown that the presence of bacterial infection is the only factor that significantly influences the level of sCD163 during AD episodes. Interestingly, we found that the more severe the infection – defined by the presence of organ failure(s) – the higher the level of sCD163. This finding is in agreement with the results of the early study of *Møller et al.* [12], in which sCD163 level was significantly higher during pneumococcal infection in cases complicated by organ failure in non-cirrhotic patients.

Here, we have shown for the first time [40], that in cirrhosis the 28-day mortality was associated with increased sCD163 level (>7000 ng/mL) during bacterial infection. Almost half of the patients with high level of sCD163 died compared to only 16% of patients with low level of sCD163. Short-term mortality risk associated with high sCD163 level (HR: 2.96) was very similar to the risk (HR: 3.05) associated with high suPAR level, another macrophage activation marker, reported in the study of *Zimmermann et al.* [41]. In our study 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 leukocyte count, respectively. This finding is consistent with the observations that excessive anti-inflammatory response, represented by interleukin-10, interleukin-6 [42] or soluble tumor necrosis factor-α receptor (sTNFR) [43] and decreased monocyte HLA-DR expression [15] has a significant negative impact on survival in cirrhosis, as recently reviewed by *Albillos et al*. [44]. 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* [16].

During bacterial infection, accurate and early selection of the most vulnerable patients requiring intensive care and monitoring is of importance in clinical practice [5]. From a pathophysiological point of view including a representative biomarker of the altered anti-inflammatory pathway into a predictive model makes sense. sCD163 might be a promising candidate for this purpose based on our findings. The present study, however, had an exploratory purpose. The relatively low sample size of patients with bacterial infection (n=99), and the single centre design did not allow us to satisfy the above mentioned, but so far unmet need. Recently predictive models of cirrhosis and short-term survival were developed in multicenter cohorts with much larger patient populations [4,45]. 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 findings of our referral cohort study of patients with cirrhosis indicate that serum sCD163 levels are not useful in outpatients for predicting

complications or death; however, it may be a useful marker for the short-term stratification of patients with bacterial infection. In this patient population, high sCD163 level is a risk factor for the short-term mortality independently from both the disease severity and the extent of the pro-inflammatory response. These results further highlight the deleterious effect of the excessive anti-inflammatory response during bacterial infection.

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**Table 1.** Epidemiologic, clinical and laboratory characteristics of patients with cirrhosis

| | | All patients n=378 | Stable outpatients n=193 | Acute decompensation n=185 |
|---|---|---|---|---|
| Gender (male/female) | | 205/173 | 93/100 | 112/73 |
| Age (years)[a] | | 56 (50-64) | 55 (49-63) | 58 (51-65) |
| Etiology, n (%) | Alcohol | 244(64.5) | 99 (51.3) | 146 (78.9) |
| | Viral | 111 (29.4) | 78 (40.4) | 30 (16.2) |
| | Other | 23 (6.1) | 16 (8.3) | 9 (4.9) |
| Child-Pugh stage, n (%) | A | 135 (35.7) | 113 (58.6) | 22 (11.9) |
| | B | 147 (38.9) | 66 (34.2) | 81 (43.8) |
| | C | 96 (25.4) | 14 (7.2) | 82 (44.3) |
| MELD score[a] | | 14 (10-18) | 11 (8-14) | 16 (12-21) |
| Serum bilirubin (μmol/L)[a] | | 33 (18-70) | 26.2 (15-43) | 52.5 (26-108) |
| Serum albumin (g/L)[a] | | 32 (27-39) | 38 (32-43) | 28 (24-32) |
| INR[a] | | 1.3 (1.1-1.5) | 1.2 (1.1-1.4) | 1.4 (1.2-1.7) |
| Ascites present, n (%) | | 192 (50.8) | 61 (31.6) | 139 (75.1) |
| Hepatocellular carcinoma, n (%) | | 41 (10.8) | 22 (11.4) | 19 (10.3) |
| Co-morbidities present, n (%) | | 191 (50.5) | 91 (47.5) | 100 (54.1) |
| Co-medication present, n (%) | | | | |
| NSSB use | | | 82 (42.5) | |
| PPI use | | | 65 (33.7) | |
| Follow up time (days)[a] | | 661 (104-1563) | 939 (323-1825) | 315 (25-1292) |
| Patients with | ascites formation[b] | | 22 (16.7) | |

| | | | | |
|---|---|---|---|---|
| complications during follow-up, n (%) | variceal bleeding | | 18 (9.3) | |
| | HE | | 29 (15.0) | |
| | bacterial infection | | 59 (30.6) | |

[a] median, IQR (lowest 25%- highest 25%); [b] in patients without ascites, INR: international normalized ratio; HE: hepatic encephalopathy, NSSBs: non-selective beta-blockers, PPIs: proton-pump inhibitors

**Table 2.** Univariate (A) and multivariate (B) Cox-regression analysis of clinical parameters and sCD163 level associated with overall survival in stable outpatients (n=193)

**A**

| | **Hazard Ratio** | **95% CI** | **p value** |
|---|---|---|---|
| Age >65 years | 1.80 | 0.96-3.39 | 0.069 |
| Gender (male) | 0.66 | 0.37-1.15 | 0.141 |
| Etiology | 1.62 | 0.90-2.94 | 0.110 |
| Co-morbidities | 2.13 | 1.20-3.77 | 0.010 |
| NSSB co-medication | 1.45 | 0.83-2.53 | 0.188 |
| PPI co-medication | 1.84 | 1.05-3.24 | 0.034 |
| Child A | Reference | | |
| Child B | 3.56 | 1.93-6.57 | < 0.001 |
| Child C | 6.91 | 2.86-16.72 | < 0.001 |
| Ln(serum sCD163 level) | 1.72 | 1.15-2.57 | 0.008 |

**B**

| | **Hazard Ratio** | **95% CI** | **p value** |
|---|---|---|---|
| Age >65 years | 1.70 | 0.85-3.42 | 0.135 |
| Gender (male) | 0.63 | 0.35-1.11 | 0.111 |
| Co-morbidities | 2.22 | 1.24-3.97 | 0.007 |
| PPI co-medication | 1.50 | 0.84-2.67 | 0.167 |
| Child A | Reference | | |
| Child B | 3.49 | 1.89-6.47 | <0.001 |
| Child C | 7.80 | 3.20-19.07 | <0.001 |
| Ln(serum sCD163 level) | 1.38 | 0.91-2.08 | 0.127 |

CI: confidence interval, NSSB: non-selective beta-blocker, PPI: proton-pump inhibitor

**Table 3.** Univariate and multivariate Cox-regression analysis for the association of risk factors of short-term mortality in patients with infection (n=99)

| | | | | **Univariate Model** | | **Multivariate Model** (Backward LR) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Variables | | 28-day mortality % (n) | | HR (95%CI) | p-value | HR (95% CI) | p-value | LRT $\chi^2$ | p-value |
| **Age (years)** | ≤65 | 25 | (18/72) | ref. | | | | | |
| | >65 | 26 | (7/27) | 0.99 (0.41-2.38) | 0.99 | | | | |
| **Gender** | female | 21 | (9/43) | ref. | | | | | |
| | male | 29 | (16/56) | 1.40 (0.62-3.18) | 0.42 | | | | |
| **Etiology** | alcohol | 20 | (16/81) | ref. | | | | | |
| | other | 50 | (9/18) | 3.18 (1.40-7.22) | 0.006 | 4.18 (1.6-10.94) | 0.004 | 7.98 | 0.005 |
| **Co-morbidities** | absent | 15 | (7/48) | ref. | | | | | |
| | present | 35 | (18/51) | 2.67 (1.12-6.40) | 0.03 | 4.48 (1.57-12.8) | 0.005 | 8.84 | 0.003 |
| **Ascites** | absent | 25 | (3/12) | ref. | | | | | |
| | present | 25 | (22/87) | 0.97 (0.29-3.24) | 0.96 | | | | |
| **AD in previous 6 months** | absent | 21 | (12/58) | ref. | | | | | |
| | present | 32 | (13/41) | 1.53 (0.70-3.35) | 0.29 | | | | |
| **MELD score**[a] | ≤21 | 10 | (7/68) | ref. | | | | | |
| | >21 | 57 | (17/30) | 7.46 (3.1-18.1) | <0.001 | 5.27 (2.08-13.3) | <0.001 | 13.65 | <0.001 |
| **CRP (mg/L)** | ≤30 | 8 | (4/48) | ref. | | | | | |
| | >30 | 41 | (21/51) | 6.03 (2.06-17.6) | 0.001 | 2.08 (0.5-8.53) | 0.31 | 1.11 | 0.29 |
| **sCD163 (ng/mL)** | ≤7000 | 17 | (11/61) | ref. | | | | | |
| | >7000 | 42 | (14/33) | 3.04 (1.38-6.71) | 0.006 | 2.96 (1.27-6.95) | 0.012 | 6.23 | 0.013 |
| **Leukocyte count**[b] | | | | 1.09 (1.03-1.15) | 0.004 | 1.12 (1.04-1.20) | 0.003 | 8.26 | 0.004 |

[a] MELD score was not available in 1 patient, due to missing albumin and creatinine level.

[b] Hazard ratio was calculated for 1G/L increase in leukocyte count

CI: confidence interval, LRT: likelihood ratio test, $\chi^2$: Chi-square, ref.: reference category, NSSBs: non-selective beta-blockers, PPI: proton-pump inhibitors

**Figure 1. Serum sCD163 levels in stable outpatients with cirrhosis according to the presence of portal hypertension.** Median sCD163 levels are not different significantly in patients between with or without (A) ascites, (B) prior variceal bleeding or esophagal varices*. Lines denote median values, boxes represent 25-75th percentiles and whiskers indicate the 5-95th range. *P* values were calculated by Mann-Whitney U-test.

* only patients screened for esophagal varices by esophago-duodenoscopy within 2 years around the study enrolment were included.

**Figure 2. Serum sCD163 levels in patients with acute decompensation event according to presence or absence of bacterial infections.** Median sCD163 levels are significantly higher in patients with infection as compared to those without. Lines denote median values, boxes represent 25-75th percentiles and whiskers indicate the 5-95th range. *P* values were calculated by Kruskal-Wallis test and Dunn's post hoc test as appropriate.

**Figure 3. Kaplan – Meier survival plot of short-term mortality in patients with acute decompensation.** (A) In patients with bacterial infection, 28-day mortality rate is higher in patients with serum sCD163 level > 7000ng/ml, compared to those with lower level. (B) In patients without bacterial infection there is no difference in 28-day mortality rate according to sCD163 level.

**Figure 4. Relationship between the estimated 28-day mortality rate and leukocyte count according to MELD score and sCD163 level.** Cut off values were >21 for MELD and >7000 ng/ml for sCD163 by Youden-index.