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Diagnostic role of CD64 expression on neutrophils as biomarker for blood stream infection in liver cirrhosis: some preliminary findings

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SUMMARY

Background: The expression of CD64 on neutrophils (nCD64), measured using flow cytometry, has been proposed as a biomarker for bloodstream infections (BSI). However, data regarding its use in the setting of liver cirrhosis are lacking.
Methods: We compared nCD64 levels in 15 cirrhotic patients with BSI to those in 19 controls, including outpatients with stable decompensated cirrhosis without infection. Additionally, we compared nCD64 with C-reactive protein (CRP) and procalcitonin (PCT) in infected hospitalized cirrhotic patients.
Results: Cirrhotic patients with infection had higher levels of nCD64 compared to controls (6.0 [5.4-7.1] vs. 2.0 [1.5-2.2]; p<0.001). Among infected patients, a correlation between nCD64 (AUC=0.934 [0.875-0.982 95% CI]), CRP (AUC=0.972 [0.942-0.993 95% CI]), and PCT (AUC=0.859 [0.739-0.953 95% CI]) was observed. However, in our sample of cirrhotic individuals, nCD64 values were not significantly different between patients with worse prognosis and those with positive outcomes (p=0.448), and its expression was not influenced by Gram stain.
Conclusions: In our cohort, nCD64 appears to be a promising new biomarker for BSI. Additional prospective studies are needed to confirm its role and limitations in conjunction with other biomarkers and rapid microbiology in the diagnostic multidisciplinary pathway for septic cirrhotic patients.

Keywords: liver cirrhosis, blood stream infection, biomarkers, CD64 expression on neutrophils, teamwork, mortality.

INTRODUCTION

Bloodstream infections (BSI) are frequently diagnosed in hospitalized patients with cirrhosis and represent the most significant precipitating event for acute decompensation, organ failure, and increased mortality [1, 2]. Portal hypertension, bacterial translocation, systemic inflamma-

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tion, and cirrhosis-associated immune and mitochondrial dysfunction are involved in the pathogenesis of infection in cirrhotic patients [3].
Considering the issues of antimicrobial stewardship and rectal colonization by multidrug-resistant organisms in cirrhotic subjects, laboratory diagnosis of infection in cirrhosis is crucial but challenging [4]. Conventional criteria often have several limitations, especially among the sickest patients. Leukocytes may be qualitatively and quantitatively influenced by hypersplenism; C-reactive protein (CRP) might reflect the underlying chronic inflammatory state rather than an infection; procalcitonin (PCT) could be falsely elevated in cases of renal dysfunction or in superimposed conditions, such as acute-on-chronic liver failure. Additionally, the time to positivity from blood cultures with antibiogram is traditionally variable, usually ≥36 hours. An exception is represented by rapid microbiology assays, which are not available in all laboratories and can fail to detect all pathogens. Therefore, rapid biochemical identification of infection, in the context of the so-called 1-hour bundle, is critical for planning treatment to reduce mortality and improve outcomes.
Among biomarkers of bacterial infection in liver cirrhosis, presepsin, pro-adrenomedullin, and interleukin-6 have shown contrasting diagnostic results in clinical practice [5-7]. However, these laboratory tests do not appear to be widely usable or available in all Hub and Spoke Hospitals.
Neutrophil CD64 (nCD64), measured using flow cytometry, has been proposed as a biomarker of sepsis. Neutrophilic granulocytes express the Fcγ receptor (FcγR) [cluster of differentiation 64 (CD64) antigen] only when activated. Its expression is strongly upregulated in response to pro-inflammatory cytokines of infection within 4-6 hours, and neutrophil CD64 integrates functions involving both innate and adaptive immune responses. Several studies have indicated that nCD64 is a highly sensitive and specific marker for the diagnosis of sepsis in many patient categories. Some authors have identified nCD64 as a biomarker in ascitic fluid of cirrhotic patients to quickly identify bacterial infections [8, 9]. However, in the context of liver cirrhosis, clinical experiences regarding the use of peripheral blood nCD64 as a predictor of BSI are scarce.
Therefore, the aim of our study is to measure peripheral blood nCD64 in a cohort of hospitalized infected patients with cirrhosis to evaluate its diagnostic role for BSI. Firstly, we compared nCD64 in cirrhotic patients with documented infection to nCD64 in controls, including outpatients with stable decompensated cirrhosis without infection. Secondly, we compared nCD64 with CRP and PCT in infected hospitalized cirrhotic patients.

MATERIALS AND METHODS

This was a prospective, observational study conducted at Hub Pordenone Hospital and Spoke San Vito al Tagliamento Hospital, Italy. Cirrhotic patients with acute decompensation (AD) associated with BSI admitted to Internal Medicine Units between June 1, 2023, and November 31, 2023, were prospectively evaluated.
Exclusion criteria included: patients ≤18 years old, absence of a definitive diagnosis of cirrhosis based on conventional histological, radiological, and biochemical criteria, and previous solid organ transplantation (including liver transplantation).
AD was defined as any first or recurrent grade 2 or 3 ascites within less than 2 weeks, first or recurrent acute hepatic encephalopathy in patients with previously normal consciousness, acute gastrointestinal bleeding, and any type of acute bacterial infection [3]. Acute bacterial infections without BSI were excluded.
For each patient, the following variables were evaluated at the time of hospital admission: age, gender, and etiology of liver disease. Acute-on-chronic liver failure (ACLF) was defined according to EASL-CLIF criteria [10]. Each patient underwent baseline measurement of blood biomarkers (blood count, CRP, PCT, nCD64). Blood and urine cultures were analyzed for all hospitalized patients. Microbiological culture with polymorphonuclear cell (PMN) count of ascitic fluid was collected in patients with ascites. The Model for End-Stage Liver Disease adding serum sodium (MELD-Na) and Child-Pugh scores were recorded at admission.
Outcomes of BSI and the length of hospital stay for each patient were recorded. Controls, including outpatients with stable decompensated cirrhosis (SDC) without infection, were also enrolled. SDC was diagnosed using commonly adopted criteria described elsewhere [3]. BSI was diagnosed using commonly adopted criteria [11]. Bacterial strains were classified according to Gram stain.

A one-step whole blood flow cytometry assay measuring CD64 mean fluorescence intensity (MFI) using IOTest Myeloid Activation (Beckman Coulter) was performed. Results were expressed as the ratio of CD64 MFI expression on neutrophils to lymphocytes (nCD64). As reported [12], a cutoff point greater than or equal to 4.59 was used to rule in bacterial infections.
All patients provided informed consent for laboratory and microbiological analysis before participation. Informed consent for using personal clinical data for study purposes was collected from all participants.

Statistical analysis
Statistical analysis was performed using MedCalc version 9.3.9.0 (Mariakerke, Belgium). Continuous variables were reported as value and percentage or median, as appropriate. The Mann-Whitney U-test was employed to compare continuous variables and significance was set at $p<0.05$. The diagnostic accuracy of nCD64, CRP, PCT, and leukocytes in predicting BSI was assessed by the area under the curve (AUC), identified by the receiver operating characteristic (ROC). AUCs were estimated with the 95% confidence interval (CI) obtained using the Wald method and compared by chi-square test.

RESULTS

We evaluated 15 cirrhotic hospitalized patients with BSI and 19 cirrhotic outpatients without signs of infection.

Study population
The characteristics of the patients enrolled are presented in Table 1. Patients with BSI at admission had similar demographic characteristics compared to those without BSI, but they were sicker in terms of liver dysfunction (Child-Pugh score: 9.9±1.8 vs. 7.8±1.3; $p<0.001$).
Among patients with BSI, AD manifested as worsening or new appearance of ascites in 12 patients (80%), acute hepatic encephalopathy in 4 patients (26.6%), and jaundice in 10 patients (66.6%). Fever was absent in 4 patients (26.6%). Any degree of acute kidney injury (AKI) was registered in 6 patients (40%), while respiratory failure was documented in one patient. Summarizing, ACLF was present in 26.6% of patients with BSI.
The most common sources of infection and Gram strains are described in Figures 1 and 2. In our cohort, Gram-positive and Gram-negative bacteria were equally represented, with a prevalence of abdominal infections (66.6%). We did not isolate any multidrug-resistant (MDR) bacteria, consistent with our favorable local epidemiology (Extended-Spectrum Beta-Lactamases Enterobacteriaceae 11.8%, Methicillin-Resistant *Staphylococcus aureus* 16.5%, Carbapenem Resistant Enterobacteriaceae 0.5% of the total isolates).
Positive urine cultures were confirmed in 2 patients; one patient had a positive ascitic culture, and another had a positive synovial fluid culture. In these patients, blood cultures detected the same pathogen as in the urine, ascitic, and synovial cultures.
Cirrhotic patients with BSI had an average length of hospital stay of 23 days, and 40% of patients were admitted to the Intensive Care Unit.
The in-hospital outcomes of infected patients were as follows: alive with antibiotic treatment (n=7), alive with antibiotic treatment and surgery (n=6), and deceased (n=2) (13.3%). Surgery was necessary due to poor infection control. Specifically, we registered: 2 laparoscopic cholecystectomies for acute gangrenous lithiasic cholecystitis, 1 heart surgery for endocarditis on the native aortic valve, 1 knee arthroscopy for septic arthritis, 1 endoscopic retrograde cholangio-pancreato-graphy (ERCP)

Table 1 - Characteristics of patients. BSI, blood stream infection, MELD-Na, Model for End-Stage Liver Disease adding serum sodium.

	BSI (n=15)	*No BSI (n=19)*	*p-Value*
Male gender, n (%)	13 (86.7%)	14 (73.7%)	0.426
Age, years	64.2±16.5	63.9±11.4	0.960
Alcoholic liver disease, n (%)	6 (40%)	7 (36.8%)	0.999
MELD-Na score	20.0±8.0	15.3±4.8	0.058
Child-Pugh score	9.9±1.8	7.8±1.3	<0.001

Figure 1
Type of infection in the group of BSI.

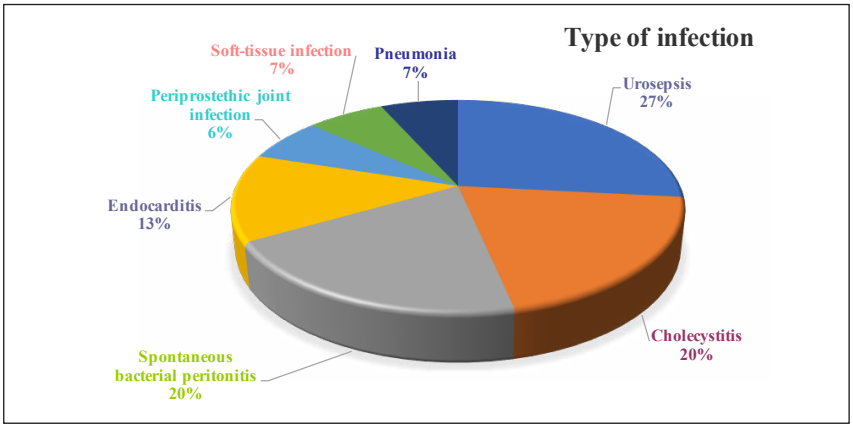
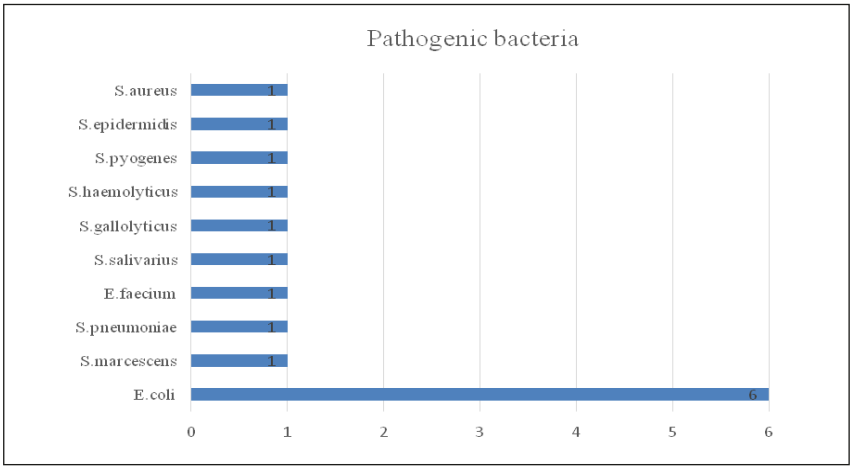


Figure 2
Pathogenic bacteria in the group of BSI.



for septic cholangitis with common bile duct lithiasis, and 1 placement of a ureteral stent for urosepsis and hydroureteronephrosis. Death occurred in an 80-year-old male patient with spontaneous bacterial peritonitis and in another 80-year-old male with major co-morbidities and urosepsis.

nCD64, CRP and PCT as biomarkers of BSI

The median nCD64 MFI was significantly elevated in the BSI group compared to controls (6.0 [5.4-7.1] vs 2.0 [1.5-2.2]; p<0.001) (Table 2). Regarding serum biomarkers, the median CRP was significantly higher in the BSI group (14.5 mg/dl [10.2-16.6]

Table 2 - WBC, white blood cell count; Hb hemoglobin, PLTs, platelets; CRP, C-reactive protein; PCT, procalcitonin; MFI, median fluorescence intensity.

	BSI (n=15)	*No BSI (n=19)*	*p-Value*
WBC, /mmc	8451±6618	3473±1780	0.012
Hb, g/dl	11.0±1.6	11.9±1.7	0.119
PLT, /mmc	108333±96,803	90263±52,404	0.522
CRP, mg/dl	14.5 (10.2-16.6)	0.5 (0.3-0.8)	<0.001
PCT, microg/L	2.33 (0.57-10.34)	0.12 (0.09-0.31)	<0.001
CD64 MFI ratio of granulocytes to lymphocytes median	6.0 (5.4-7.1)	2.0 (1.5-2.2)	<0.001

Table 3 - Area under the ROC curve of predictors of BSI occurrence.

	AUC-ROC	*95%CI*
Neutrophils, /mmc	0.770	0.599-0.904
Log CRP, mg/L	0.972	0.942-0.993
Log PCT, mcg/L	0.859	0.739-0.953
Log CD64 MFI ratio	0.934	0.875-0.982

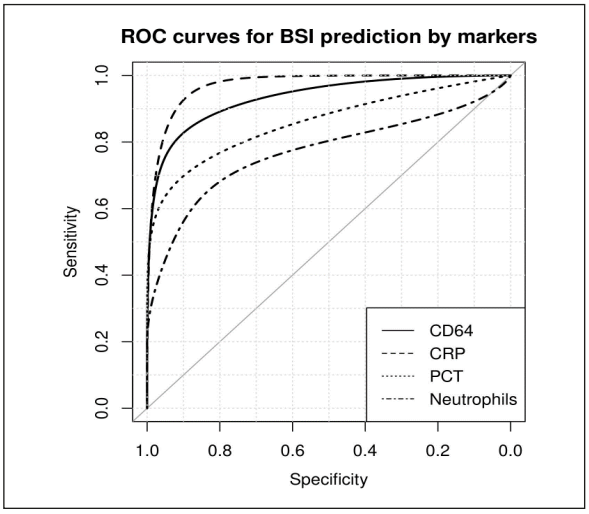


Figure 3 - ROC curves of predictors of BSI occurrence.

vs 0.5 mg/dl [0.3-0.8]; p<0.001), as was the median PCT (2.33 µg/L [0.57-10.34] vs 0.12 µg/L [0.09-0.3]; p<0.001), and the median white blood cell count (8451±6618/mmc vs 3473±1780/mmc; p=0.012). In infected patients, a correlation among nCD64, CRP, and PCT values was demonstrated (Table 3 and Figure 3). The expression of CD64 on neutrophils was not associated with the bacterial species distinguished by Gram strain.

Prognostic role of nCD64 in BSI

In BSI group, we registered negative outcome as organ failure, mortality, poor source control infection in 11 patients (73.3%). Values of nCD64 were not statistically different in patients with worse prognosis compared to patients with positive outcome (p=0.448).

DISCUSSION

Bloodstream infection (BSI) is a critical event in the course of cirrhotic patients. In our sample, we observed a mortality rate of 13.3%, with acute-on-chronic liver failure (ACLF) recorded in 26.6% of cases, and 40% of patients requiring surgery due to inadequate infection control. Considering that the average length of hospital stay for cirrhotic patients is 11.5 days based on data from the Associazione Italiana Studio del Fegato (AISF), our real-world results showed a doubling of hospitalization duration for BSI in liver cirrhosis, leading to increased healthcare costs [13].

Consequently, a multidisciplinary approach involving hepatologists, intensivists, infectious disease specialists, expert laboratorists, and microbiologists is essential to achieve prompt laboratory diagnosis of BSI and early, appropriate treatment to reduce morbidity and mortality in these patients.

To our knowledge, this study is the first to report on the use of peripheral blood nCD64 as a biomarker compared to CRP and PCT for predicting BSI in cirrhotic patients. In our sample, nCD64 levels in septic patients were significantly elevated compared to the control group. This finding is consistent with previous studies that have documented higher neutrophilic granulocyte counts in infected patients. Similarly, we confirmed that procalcitonin is a useful biomarker for diagnosing infections in cirrhotic patients [14].

Based on these data, clinicians may be able to identify infectious sepsis using nCD64 before obtaining pathogen culture results. A diagnostic algorithm incorporating nCD64 could be proposed to support clinical decision-making. nCD64 is a simple test with a short turnaround time (1-2 hours) and has a cost comparable to that of C-reactive protein and procalcitonin. This test can be performed in any laboratory with flow cytometry facilities and does not require special equipment or expertise, although each laboratory would need to establish its own cutoff values. These characteristics make nCD64 an attractive test to incorporate into daily clinical practice, particularly for the admission of cirrhotic patients with acute decompensation in the Emergency Department, where hospitals are often overcrowded and beds are in short supply. A rapid laboratory diagnosis of sepsis using nCD64 and other biomarkers could exemplify a lean thinking program, including correct allocation of septic patients (intensive care vs. ordinary care), diagnostic imaging, and empiric broad-spectrum antibiotic therapy with subsequent reasoned therapeutic de-escalation.

Our findings indicate that nCD64 is not a good predictor for estimating the severity of sepsis and mortality in cirrhotic patients. Given that our results are based exclusively on liver cirrhosis, it is difficult to compare these data with other studies. However, in general, some authorshave concluded that nCD64 is a prognostic marker of sepsis in critically ill patients [15, 16].

There are several limitations to our study. Firstly, we studied a small sample size. Secondly, we focused on BSI, which is a subgroup of infections. Additionally, due to the small sample size, it was not possible to conduct a subgroup analysis for different individual microorganisms.

In our opinion, it was necessary to demonstrate the diagnostic accuracy of nCD64 in a context of proven and well-documented infection such as BSI. According to our data, WBC, CRP, and PCT perform well as sepsis markers in cirrhosis, but it must be noted that the values of these markers are affected by sepsis itself and could vary in different types of infections that do not involve sepsis. Furthermore, we did not assess the Sequential Organ Failure Assessment (SOFA) score.

In conclusion, neutrophil CD64 could be a new laboratory tool for assessing BSI in cirrhosis. Our future challenge is to confirm our preliminary results on a larger population of cirrhotic patients with infections in other settings beyond BSI.

Competing interests

No conflict of interest must be declared for any of the authors.

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