Study of CD64 and HLA-DR as Early Detection Markers of Sepsis in Hepatic ICU Patients

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Background and study aim: Sepsis is the most common cause of death in liver cirrhosis patients. Aim: Evaluation of neutrophil and monocyte CD64 and HLA-DR as early biomarkers predicting sepsis in liver cirrhosis.

Patients and Methods: This case-control study involved 70 cirrhotic patients (35 with sepsis and 35 without) and 30 healthy individuals. Laboratory studies were performed, including CD64 and HLA-DR using flow cytometry along with sepsis index (SI).

Results: Patients were mainly males (80%), aged 62.17 ± 7.56, and 64.69 ± 11.64 years in group 1&2 respectively. Mono CD64% at Cut-off: >62.9 showed AUC: 0.676, Sensitivity: 80.0%, Specificity: 60.0%, PPV: 66.7%, and NPV: 75.0%, while Mono CD64 MFI at Cut-off >9.5 showed AUC: 0.659, Sensitivity: 94.29%, Specificity: 37.14%, PPV: 60.0%, and NPV: 86.7%. Combinations of CRP+ Lactate+ Mono CD64%+ Mono revealed AUC: 0.929, Sensitivity: 82.86%, Specificity: 91.43%, PPV: 90.6%, and NPV: 84.2% Mono CD64% and CD64 MFI (0.003; 0.03] respectively.

Regression analysis defined Mono CD64% >62.9 (OR 6], Mono CD64 MFI >9.5 (OR 9.75) CRP >32.4 (OR 13.5), lactate>1.93 (OR 12.08) and ALBI score>0.01 (OR 6) all as factors affecting early sepsis in cirrhosis.

Conclusion: Mono CD64% and Mono CD64 MFI proved efficacy as early septic biomarkers with higher efficacy when combined with traditional inflammatory markers in liver cirrhosis patients.

INTRODUCTION

In intensive care units (ICUs), sepsis stands as a leading cause of morbidity and mortality, posing diagnostic challenges due to diverse comorbidities and underlying illnesses [1]. Globally, sepsis, responsible for 48.9 million cases and 11.0 million deaths in 2017, remains a critical health concern, necessitating immediate attention, particularly in terms of early detection and innovative therapeutic approaches [2].Traditional diagnostic methods for sepsis, relying on serum analysis and molecular techniques, confront challenges due to vague symptoms and a lack of a definitive gold standard test for confirmation [3]. Blood culture tests, commonly used for identifying infectious bacteria, are time-consuming and not always accurate. Molecular methods, encompassing polymerase chain reaction and microarray, offer varying sensitivity and specificity. Despite the development of over 170 biomarkers for sepsis screening, only a few prove significant in practical applications [4]. Cirrhosis was known as a precipitant of sepsis due to Bacterial overgrowth, increased intestinal permeability, and cirrhosis-associated immune dysfunction [CAID] predispose cirrhotic patients to bacterial infections, which in turn leads to

four-fold increased mortality compared with non-cirrhotic patients [5].

Nevertheless, the need for early predictors of the occurrence of sepsis and mortality in patients with liver cirrhosis was only conducted by investigating various immune cell markers, including PMN CD64%, PMN CD64 MFI, PMN HLA-DR%, Mono CD64%, Mono CD64 MFI, Mono HLA-DR MFI, and Sepsis Index CD64, a high-affinity Fcγ receptor, exhibits heightened expression on neutrophils during systemic inflammatory response syndrome [SIRS], making it a promising early marker for bacterial infection [6].

Correspondingly, reduced monocytic HLA-DR (mHLA-DR) expression serves as a consistent marker for immunosuppression in sepsis patients [7].

Flow cytometry (FCM) emerges as a valuable diagnostic tool for immune-related disorders, offering insights into the systemic response to infection through profiling cytokines and surface markers (e.g., neutrophil CD64 and mHLA-DR). This was the impulse of the current study assessing the role of neutrophil CD64 and HLA-DR as markers of early detection of sepsis in liver cirrhosis patients.

PATIENTS/MATERIALS AND METHODS

Patients:

This case-control study was conducted on patients diagnosed with liver cirrhosis, either complicated with sepsis or not. Participants were recruited from outpatients, inpatients, and the Intensive Care Unit [ICU] within the Hepatology and Gastroenterology Department at the National Liver Institute, Menoufia University. Patients with cirrhosis were diagnosed based on clinical, laboratory, and radiological criteria [8].

Sepsis diagnosis in patients was based on clinical suspicion (cultures taken or antibiotics started] along with the fulfillment of SIRS criteria (2 or more of White Cell Count (WCC) >11 or <4, Heart Rate (HR) >90, Respiratory Rate (RR) >20, or temperature >38 or <36°C) [9-11].

Exclusion criteria

Participants meeting any of the following criteria: Patients younger than 18 years, acute pancreatitis, septic shock at the time of enrolment, severe organ failure at the time of enrolment (an immediate requirement for ventilation, vasopressor, or renal replacement therapy), hematological malignancy, recent chemotherapy (within the past 2 weeks), myelodysplastic syndromes, known neutropenia, pregnancy, blood transfusion exceeding 4 units in the past week, oral corticosteroids for >24 hours prior to enrolment, patients with human immunodeficiency virus [HIV] or patients with an ICU length of stay less than 24 Hours or more than 100 days were excluded.

Patients were categorized into the following groups: Group 1: 35 ICU Cirrhotic patients with sepsis meeting inclusion criteria, Group 2: 35 cirrhotic patients without sepsis and Group 3: 30 healthy individuals without a history or clinical evidence of liver disease or any other disease, with negative anti-HCV and HBsAg.

For all patients, the following procedures were conducted: History taking [age, sex, history of blood transfusion, history of any surgery], Clinical examination, Chest X ray, Abdominal sonar, Laboratory investigations, including (Complete blood count [CBC], CRP, kidney function test, Urine analysis, Anti-HCV and detection of HCV RNA by PCR, HBV serological markers [HBsAg and anti-HBc], Liver profile: alanine aminotransferase [ALT], aspartate aminotransferase [AST], serum albumin, total bilirubin, ascitic fluid analysis for spontaneous bacterial peritonitis (12), and Serial measures of serum lactate.

Sepsis index (based on the combination of two CBC parameters: monocyte distribution width (MDW) and mean monocyte volume (MMV) [14] (The Sepsis Index Score from two monocyte parameters often refers to a method that evaluates sepsis risk using monocyte distribution width (MDW) and monocyte volume distribution width (MVW). SI=MDW×MVW/100.

1. MELD−Na−[0.025×MELD×(140−Na)]+14 000) [14], ALBI score (log10 bilirubin×0.66)+(albumin×−0.085) [15].
2. Culture and antibiotic sensitivity testing.
3. Detection of PMN CD64%, PMN CD64 MFI, PMN HLA-DR%, PMN HLA-DR MFI, Mono CD64%, Mono CD 64 MFI, Mono HLA-DR%, and Mono HLA-DR MFI using flow cytometry [16, 17].

Sampling

The antibody used in this study underwent quality control testing through immunofluorescent staining with flow cytometric analysis. The recommended amount of reagent was 5 µL of antibody per test [for a million cells in 100 µL staining volume or per 100 µL of whole blood].

Ethical Approval

The study protocol received approval from the ethical committee of our institution, and all selected patients provided informed consent before enrolment in the study.

Statistical analysis

The data was first coded and verified before its entry. The computer program Statistical Package for Social Sciences (SPSS) (ver.21) Chicago, USA was used for analyzing the collected data and for drawing figures.

Data expressed as mean ± standard deviation and number, percentage. Student-t-test and ANOVA tests were used to determine the significant difference for the numeric variable. Chi.-square was used to determine the significant values for categorical variables. Person's correlation was used for correlations between groups. P value is considered significant when P value < 0.05 and not significant when P value > 0.05.

RESULTS

All demographic and laboratory criteria are illustrated in **table 1**. The most common causes of infections in liver cirrhosis patients were mainly spontaneous bacterial peritonitis (SBP) **(table 2).**

CRP at Cut-off Point: >50 showed AUC: 0.882, Sensitivity: 80.0%, Specificity: 77.14%, with positive predictive value (PPV): 77.8%, and negative predictive value (NPV): 79.4%.

Lactate at Cut-off Point: >3.5 showed AUC: 0.750, Sensitivity: 71.43%, Specificity: 82.86%, PPV: 80.6%, and NPV: 74.4%. Mono CD64% at Cut-off Point: >62.9 showed AUC: 0.676, Sensitivity: 80.0%, Specificity: 60.0%, PPV: 66.7%, and NPV: 75.0%.

Mono CD64 MFI at Cut-off Point >9.5 shoed AUC: 0.659, Sensitivity: 94.29%, Specificity: 37.14%, PPV: 60.0%, and NPV: 86.7%.

Patients with sepsis showed that the combination of ALBI score and S. Lactate had sensitivity, specificity, PPV, NPV, and accuracy of 80%, 80%, 80%, 80%, and 0.831%, respectively **(figure 1D).**

Combinations of CRP + S. Lactate + Mono CD64% + Mono CD64 MFI revealed AUC: 0.929, Sensitivity: 82.86%, Specificity: 91.43%, PPV: 90.6% and NPV: 84.2% **(figure 1).**

The immune markers only Mono CD64% and Mono CD64 MFI were significantly elevated in septic patients than others (P=0.01) **(table 3).**

According to Uni-Varity model, early prediction of sepsis in cirrhotic patients we noticed the following results: (Creatinine >1 with OR 4.231 – Urea >107 with OR 8 – Na ≤136 with OR 7.222 - T.B >3.29 with OR 8 – PMN >68.1 with OR 0.120 – Lymphocytes ≤8.4 with OR 11.625 - Mono CD64% >62.9 with OR 6 - Mono CD64 MFI >9.5 with OR 9.750 – CRP >32.4 with OR 13.500 – Lactate >1.93 wit OR 12.083 - ALBI score >0.01 with OR 6 - MELD (UNOS)>7.66 WITH OR 22 - MELD-Na >10.53 with OR 13.5).

According to Muli-Varity model, early prediction of sepsis in cirrhotic patients showed the following: (CRP >32.4 with OR 67.014, Lactate >1.93 with OR 128.345, ALBI score >0.01 with OR 46.461, MELD [UNOS) >7.66 with OR 21.709, and MELD-Na >10.53 with OR 21.184); this is summarized in **table 4**.

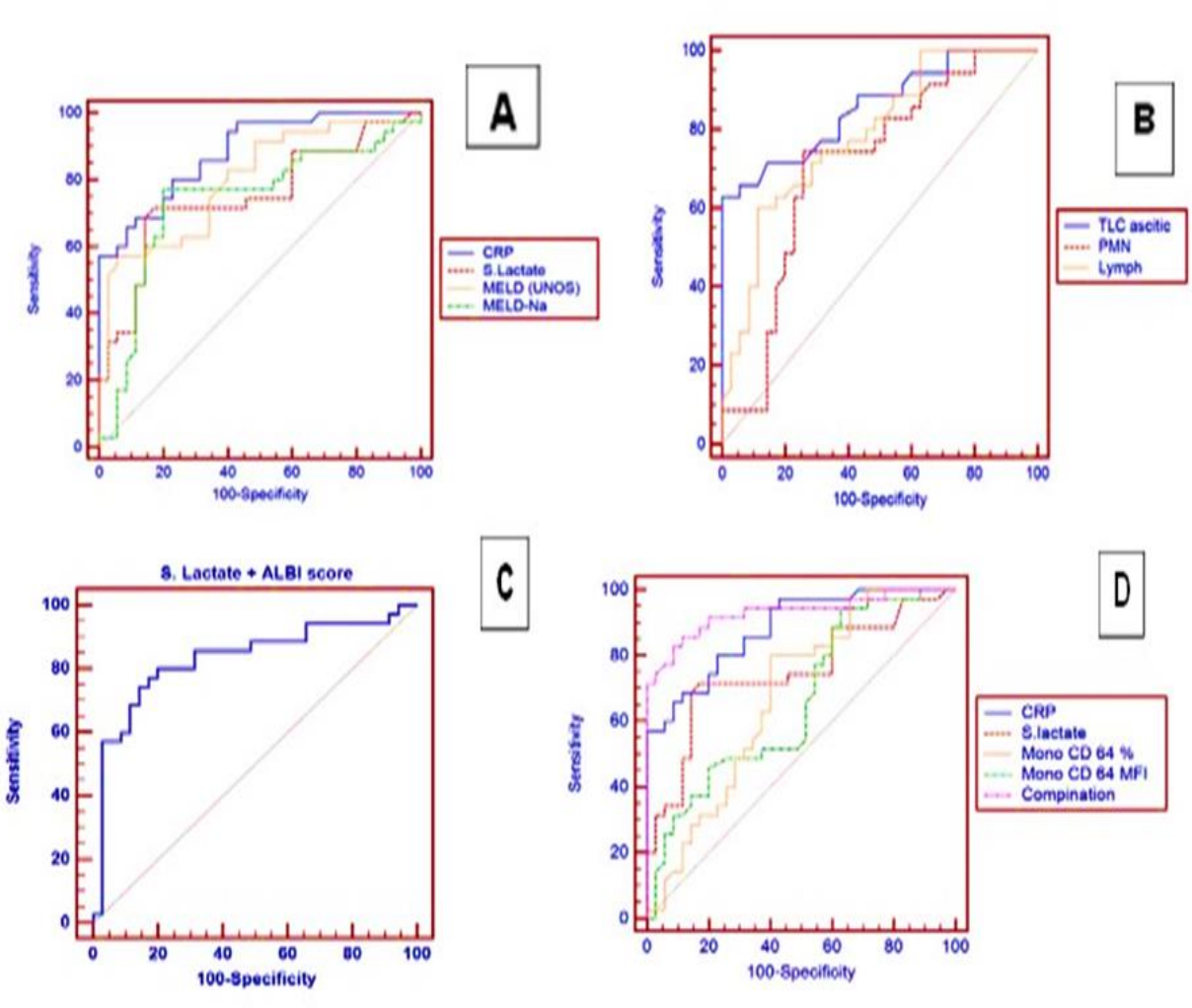

Figure 1. ROC curves of factors predicting early sepsis: A: CRP-Lactate-MELD- MELD Na- B: TLC-PMN- Lymphocytes, C: ALBI score, D: ALBI Lactate, D:. CRP, S. Lactate, Mono CD64%, and Mono CD64 MFI.

Table 1. Comparison between the two studied groups of cirrhotic patients regarding demographic and laboratory parameters

		Group 1	Group 2	Test value	P-value	Sig.
		No. = 35	No. = 35			
Age	Mean ± SD	62.17 ± 7.56	64.69 ± 11.64	-1.072•	0.288	NS
	Range	49 - 75	43 - 87			
Sex	Females	7 [20.0%]	11 [31.4%]	1.197*	0.274	NS
	Males	28 [80.0%]	24 [68.6%]			
HB	Mean ± SD	10.09 ± 1.77	10.12 ± 2.51	-0.061•	0.952	NS
	Range	6.6 - 13.3	6.1 - 16.9			
TLC	Median [IQR]	10.5 [6.2 - 16.6]	6.5 [5.2 - 9.3]	-3.478≠	0.001	HS
	Range	4.8 – 45.2	4.4 – 12.4			
PLT	Median [IQR]	125 [73 - 197]	115 [69 - 167]	-0.628≠	0.530	NS
	Range	25 – 459	39 – 416			
Creatinine	Median [IQR]	1.47 [0.95 - 2.73]	0.98 [0.69 - 1.39]	-2.967≠	0.003	HS
	Range	0.6 – 5.08	0.26 – 3.09			
Urea	Median [IQR]	123 [51 - 185]	58 [33 - 85.5]	-2.843≠	0.004	HS
	Range	18 – 350	14 – 233			
Na	Mean ± SD	133.54 ± 7.08	137.17 ± 6.25	-2.273•	0.026	S
	Range	113 – 151	113 - 144			
K^+	Mean ± SD	4.3 ± 0.82	4.33 ± 1.19	-0.128•	0.898	NS
	Range	3 - 6.1	1.2 - 9.9			
ALT	Median [IQR]	33 [25 - 97]	30 [19 - 50]	-1.445≠	0.148	NS
	Range	11 – 463	11 – 129			
AST	Median [IQR]	70 [37 - 154]	49 [31 - 97]	-1.410≠	0.159	NS
	Range	13 – 1575	18 – 419			
Alb	Mean ± SD	2.65 ± 0.54	2.82 ± 0.74	-1.139•	0.259	NS
	Range	1.5 - 3.7	1.3 - 4.4			
T.B	Median [IQR]	3.91 [1.12 – 14.38]	1.6 [0.9 – 2.71]	-2.402≠	0.016	S
	Range	0.13 - 31.91	0.23 - 29			
ALBI score	Median [IQR]	0.19 [-0.37 – 0.74]	-0.23 [-0.49 – 0.01]	-2.391≠	0.017	S
	Range	-1.36 – 1.14	-1.11 – 1.07			
MELD [UNOS]	Median [IQR]	7.76 [7.26 – 8.44]	6.92 [6.48 – 7.57]	-4.282≠	0.001	HS
	Range	5.54 – 10.12	5.82 – 8.82			
MELD-Na	Median [IQR]	11.81[10.6 – 17.12]	8.46 [6.22 – 10.39]	-3.418≠	0.001	HS
	Range	-0.83 – 29.36	2.62 – 29.1			

P>0.05: Non-significant [NS]; P <0.05: Significant [S]; P <0.01: Highly significant [HS], HB: hemoglobin, TLC: total leucocytic count, PLT: platelets, Na: sodium, K: potassium, ALT: alanine transaminase, AST: aspartate transaminase, ALB: albumin, T.B.: total bilirubin, ALBI score:: albumin bilirubin score, MELD [UNOS]: model of end stage liver disease, MELD-Na: model of end stage liver disease Na
•: Independent t-test; ≠: Mann Whitney test

Table 2. Comparison between the two studied groups of cirrhotic patients regarding the source of infection and inflammatory markers.

		Group 1 [Sepsis] (35)	**Group 2 [Non-sepsis]** (35)	**Test value**	**P-value**	**Sig.**
Urine (pus cell)	Median[IQR] Range	5 [2 - 10] 1 – 55	3 [2 - 7] 2 – 15	-1.449‡	0.147	NS
CXR	Negative Positive	27 [77.1%] 8 [22.9%]	35 [100.0%] 0 [0.0%]	9.032*	0.003	HS
TLC ascitic	Median[IQR] Range	0.9 [0.34 - 4.2] 0.1 – 23.43	0.25 [0.09 - 0.36] 0.02 – 0.45	-5.122‡	0.001	HS
PMN	Mean ± SD Range	69.97 ± 15.65 30.9 - 94.9	54.66 ± 22.02 12.8 - 88.9	3.353•	0.001	HS
Lymphocyte	Median [IQR] Range	8.1 [4.8 - 17.7] 1 – 36.1	23.5 [10.9 - 45.60] 3.7 – 80.4	-4.088‡	0.001	HS
Monocyte	Median [IQR] Range	7.5 [5.7 - 11.7] 1 – 18.8	8.7 [5.7 - 12.4] 2.7 – 22.7	-0.640‡	0.522	NS
CRP	Median [IQR] Range	115.3 [85.9 – 153.9] 6.3 – 311	8.8 [5 – 50] 0.8 – 97.88	-5.498‡	0.001	HS
S. Lactate	Median [IQR] Range	9.5 [1 – 25.5] 0.33 – 48.5	1.3 [0.62 - 2.5] 0.11 – 36.4	-3.595‡	0.001	HS

P>0.05: Non-significant [NS]; P <0.05: Significant [S]; P <0.01: Highly significant [HS], CRP: C reactive protein, PMN: polymorph nuclear leukocytes, TLC ascitic: total leukocytic count in ascitic, CXR: chest x-ray, IQR: inter quartile ratio. *: Chi-square test; •: Independent t-test; ≠: Mann Whitney test

Table 3. Comparison between the three studied groups regarding Neutrophil, Monocytes and sepsis index of the studied patients

		Group 1 (35)	**Group 2 (35)**	**Group 3 (30)**	**Test value**	**P-value Sig.**
PMN CD64%	Mean±SD Range	16.32 [7.97 – 30.6] 1.55 - 92.2	13.9 [8.1 – 23.4] 2.1 - 63.2	3.1 [2 – 4.8] 1.4 - 64	8.314•	0.001 HS
PMN CD64 MFI	Mean±SD Range	8.19 [6.87 – 10] 3.79 - 19.4	7.96 [6.48 – 9.93] 4.42 - 38.3	5.65 [5.11 – 6.12] 2.08 - 7.84	8.121≠	0.001 HS
PMN HLA-DR%	Mean±SD Range	5.95 [2.41 – 15.34] 0.6 - 40.96	15.72 [3.55 – 23.3] 1.46 - 56.84	3.57 [2.6 – 6.4] 0.94 - 12.19	9.828≠	0.001 HS
PMN HLA-DR MFI	Mean±SD Range	5.92 [4.04 – 9.3] 0.5 - 15.7	4.79 [3.58 – 7.75] 2.01 - 17.6	4.99 [4.37 – 7.12] 2.26 - 15.3	0.784≠	0.460 NS
Mono CD64%	Mean±SD Range	71.8 ± 14.6 39.3 – 95	58.1 ± 22.33 20.39 - 93.5	59.33 ± 14.46 6.4 - 85.4	6.325•	0.003 HS
Mono CD 64 MFI	Mean±SD Range	13.19 ± 3.23 6.76 - 20.5	11.25 ± 4.07 4.71 - 25.8	7.2 ± 2.49 3.42 - 17.2	26.365•	0.001 HS
Mono HLA-DR%	Median [IQR] Range	60.38 [50.59 - 79.46] 15.7 – 96.4	55.1 [29.29 - 73.6] 2.32 – 91	68.6 [59.6 - 78.32] 0.6 – 87	5.565≠	0.062 NS
Mono HLA-DR MFI	Median [IQR]	11.7 [10.1 - 18.1]	10.9 [6.53 - 15.9]	8.61 [7.02 - 10.7]	12.387≠	0.002

	Range	5.44 – 37.9	4.1 – 31.7	1.74 – 21.7		HS
Sepsis INDEX [SI]	Median [IQR] Range	23.73 [13.72 – 48.53] 2.65 – 288.6	28.46 [15.32 – 62.82] 5 – 594.83	5.48 [2.92 – 7.74] 1.78 – 550	35.213≠	0.001 HS
Post Hoc Analysis						
Parameters		**P1**		**P2**	**P3**	
PMN CD64%		0.573		0.001	0.001	
PMN CD64 MFI		0.526		0.001	0.001	
PMN HLA-DR%		0.068		0.049	0.001	
Mono CD64%		**0.011**		0.001	0.990	
Mono CD64 MFI		**0.022**		0.001	0.001	
Mono HLA-DR MFI		0.106		0.001	0.079	
Sepsis INDEX [SI]		0.394		0.001	0.001	

:•One Way ANOVA test; ≠: Kruskall-Wallis test
P1: Comparison between group 1 vs group 2
P2: Comparison between group 1 vs group 3
P3: Comparison between group 2 vs group 3

Table 4: Logistic regression analysis for predictors of sepsis group

	Univariate				**Multivariate**			
	P-value	**Odds ratio [OR]**	**95% C.I. for OR**		**P-value**	**Odds ratio [OR]**	**95% C.I. for OR**	
			Lower	**Upper**			**Lower**	**Upper**
Creatinine>1	**0.005**	4.231	1.550	11.546	**0.289**	**0.188**	**0.009**	**4.124**
Urea>107	**0.001**	8.000	2.509	25.507	--	--	--	--
Na≤136	**0.001**	7.222	2.515	20.736	--	--	--	--
T.B>3.29	**0.001**	8.000	2.509	25.507	**0.689**	**5.352**	**0.001**	**19899.516**
PMN>68.1	**0.001**	0.120	0.041	0.350	--	--	--	--
Lymph≤8.4	**0.001**	11.625	3.359	40.236	--	--	--	--
Mono CD64%>62.9	**0.001**	6.000	2.060	17.479	**0.198**	**5.222**	**0.421**	**64.700**
Mono CD64 MFI>9.5	**0.005**	9.750	2.001	47.498	**0.162**	**12.393**	**0.363**	**423.241**
CRP>32.4	**0.001**	13.500	4.301	42.375	**0.026**	**26.402**	**1.467**	**475.117**
S.Lactate >1.93	**0.001**	12.083	3.846	37.963	**0.007**	**65.347**	**3.159**	**1351.831**
ALBI score>0.01	**0.001**	6.000	2.060	17.479	**0.928**	**1.441**	**0.000**	**4181.903**
MELD [UNOS] >7.66	**0.001**	22.000	4.547	106.434	**0.073**	**21.709**	**0.754**	**625.417**
MELD-Na >10.53	**0.001**	13.500	4.301	42.375	**0.060**	**21.184**	**0.882**	**508.590**

DISCUSSION

Exploring the diagnostic markers of early sepsis in cirrhotic patients with CAID had emphasized the significance of cell immune markers in timely detection in the ICU setting [17].

The current study had demarcated significant elevations in ordinary sepsis markers like C-reactive protein (CRP), PMN counts, lactate, and sepsis index (P=0.001) in ICU patients with sepsis. This is the usual picture of a cirrhotic septic patient in the ICU [18]. CRP, being an acute-phase reactant produced by the liver during inflammation, is recognized as a valuable marker, known for its characteristic surge during infection, often rising significantly [18]. Additionally, D'Abrantes. et al, emphasized the predictive value of plasma lactate levels in

assessing the prognosis of sepsis [19]. Hyperlactatemia and lactic acidosis, as observed in our study, may result from increased lactate production due to impaired tissue oxygenation, stemming from reduced oxygen delivery or disorders in oxygen utilization, ultimately leading to heightened anaerobic metabolism [20].

In this study, a significantly elevated total bilirubin (TB) in septic patients compared to the non-septic cirrhotic group, pointing to the role of sepsis in this

elevation. Cholestasis-induced sepsis (CIS) differs from hepatic cellular dysfunction-associated cirrhosis in its gradual onset post-ICU admission, marked by rising bilirubin, alkaline phosphatase, and gamma-glutamyl transferase levels.

Cirrhotic patients with sepsis demonstrated elevated ALBI scores, MELD (UNOS), and MELD-Na values in comparison to non-septic cases. This observation aligns with the understanding that sepsis is a significant milestone in the progression towards mortality, and all these scores serve as reliable indicators of short-term survival [21]. Elevated bilirubin due to CIS contributes greatly to these elevations. Also, albumin, which is significantly reduced in cirrhosis-sepsis cases might be an important discriminator [22]. Hypoalbuminemia is more common in sepsis patients, particularly those with septic shock, due to the leakage of protein-rich fluid caused by capillary dysfunction. Previous research indicates that low serum albumin levels are associated with higher mortality risk in sepsis [23-24].

However, the need for early predictors of the occurrence of sepsis and mortality in patients with liver cirrhosis was only conducted by investigating various immune cell markers, including PMN CD64%, PMN CD64 MFI, PMN HLA-DR%, Mono CD64%, Mono CD64 MFI, Mono HLA-DR MFI, and Sepsis Index (SI).

Statistically significant elevations in Mono CD64%, Mono CD64 MFI, and SI were observed in cirrhotic patients, with sepsis, indicating altered immune responses and signifying their prognostic value.

The diagnostic accuracy of these markers was assessed with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy. CRP at Cut-off Point: >50 showed AUC: 0.882, Sensitivity: 80.0%, Specificity: 77.14%, with positive predictive value (PPV): 77.8%, and negative predictive value (NPV): 79.4%. Lactate at Cut-off Point: >3.5 showed AUC: 0.750, Sensitivity: 71.43%, Specificity: 82.86%, PPV: 80.6%, and NPV: 74.4%. Mono CD64% at Cut-off Point: >62.9 showed AUC: 0.676, Sensitivity: 80.0%, Specificity: 60.0%, PPV: 66.7%, and NPV: 75.0%. while Mono CD64 MFI at Cut-off Point >9.5 shoed AUC: 0.659, Sensitivity: 94.29%, Specificity: 37.14%, PPV: 60.0%, and NPV: 86.7%. Patients with sepsis showed that the combination of ALBI score and S. Lactate had sensitivity, specificity, PPV, NPV, and accuracy of 80%, 80%, 80%, 80%, and 0.831%, respectively. Combinations of CRP + S. Lactate + Mono CD64% + Mono CD64 MFI revealed AUC: 0.929, Sensitivity: 82.86%, Specificity: 91.43%, PPV: 90.6%, and NPV: 84.2%. Mono CD64 MFI (94.29%) stands out as having the highest sensitivity, which is crucial for identifying true positive cases. The combined use of CRP, S. Lactate, Mono CD64%, and Mono CD64 MFI revealed the highest AUC (0.929). Additionally, this combination had the highest specificity needed for minimizing false positives (better overall diagnostic performance).

Prior studies by Davis et al., Hsu et al., and Dal Ponte et al. have underscored the superior diagnostic performance of nCD64 over traditional markers like white blood cell count, erythrocyte sedimentation rate, and CRP [25-27]. Icardi et al. further highlighted the predictive value of nCD64 with a sensitivity of 94.6% and a specificity of 88.7% [28]. Lewis et al. and Zhou et al. emphasized the discriminatory power of CD64, particularly in distinguishing septic shock patients [29-30]. Additionally, the combination of nCD64 and CRP has been shown to enhance sepsis diagnosis [31]. Chauhan et al. advocated for flow cytometry analysis of nCD64, asserting its superiority in sepsis detection [32].

In a recent study by Verma et al., the mean fluorescence intensity (MFI) of neutrophil CD64 (nCD64) was markedly elevated in both sepsis and non-sepsis groups compared to controls, demonstrating the diagnostic potential of nCD64 [33].

In summary, the combined use of CRP, S. Lactate, Mono CD64%, and Mono CD64 MFI has a higher AUC and shows promising sensitivity, specificity, PPV, and NPV, making it a potentially effective diagnostic tool for sepsis.SO, healthcare professionals may improve their ability to identify sepsis at an early stage,

facilitating prompt and targeted interventions for improved patient outcomes.

Furthermore, predictive models based on univariate and multivariate analyses highlighted key predictors for early sepsis detection in cirrhotic patients, emphasizing the significance of factors such as creatinine, urea, sodium, total bilirubin, PMN, lymphocytes, Mono CD64%, Mono CD64 MFI, CRP, S. Lactate

In addressing the limitations of this study, such as the sample size and potential confounding factors, we delve into recommendations for future research. It is suggested that future investigations should focus on a more in-depth exploration of specific immune markers and their dynamics in larger-size studies on patients with cirrhosis.

This study is a pioneer in presenting comprehensive findings on the correlation of immune-related markers with sepsis in a cirrhotic cohort, showcasing the potential of these markers as early indicators of early sepsis management in cirrhotic patients, emphasizing the broader relevance of the findings in critical care settings.

CONCLUSION

Conclusively, compared to traditional markers like CRP and S. Lactate, CD64 has comparable diagnostic value for distinguishing sepsis in cirrhotic patients with the advantageous timely character allowing prompt management for this high-risk critical cohort.

Acknowledgments

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Abbreviations

MELD: Model for End-Stage Liver Disease, ICU: intensive care unit, CBC: Complete Blood Count, CRP: C reactive protein, PMN: polymorph nuclear leukocytes, TLC ascitic: total leukocytic count in ascitic, SI: Sepsis index, CXR: chest x-ray, IQR: inter quartile ratio, CAS: cholestasis associating sepsis.

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Author Contributions:

Conceptualization, E.M., KH.F. and A.G.; methodology, A.F., KH.F.; formal analysis, A.M., and A.M.; data curation, A.F., A.M.; writing—original draft preparation, A.F.; writing—review and editing, E.M.; supervision, A.G., A.M.; funding acquisition, A.F. All authors have read and agreed to the published version of the manuscript.

Ethical approval: The Institutional Review Board at National Liver Institute, Menoufia University determined that our study was exempt from review.

Availability of data and materials:

Data available upon request.

HIGHLIGHTS

- Sepsis is considered the most common cause of death in liver cirrhosis patients.
- This study pioneers in presenting comprehensive findings on the correlation of immune-related markers with sepsis in a cirrhotic cohort.
- The potentiality of these markers as early indicators of early sepsis management in cirrhotic patients had emphasized the broader relevance of the findings in critical care settings.
- Compared to traditional markers like CRP and S. Lactate, CD64 has comparable diagnostic value to distinguish sepsis in cirrhotic patients with the advantageous timely character allowing prompt management for this high risky critical cohort.

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