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ORIGINAL RESEARCH

Detection of Inflammatory Biomarkers Among Patients with Sepsis of Gram-Negative Bacteria: A Cross-Sectional Study

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Background: Sepsis is a highly mixed ailment that affects patients with numerous conditions of infectious sources and can lead to multi-organ failure with dysregulated host immune response.

Objective: To determine inflammatory biomarkers in patients with sepsis caused by Gram-negative bacteria and compare their role in the early detection of sepsis.

Methods: This cross-sectional study was conducted on patients with sepsis admitted to the intensive care unit at different hospitals in Sulaimaniyah, Iraq, from May to December 2021. Patients (n=147) were enrolled in this study according to the primary diagnosis of sepsis by Sequential Organ Failure Assessment scores. Blood samples were taken from patients to investigate white blood cells, inflammatory biomarkers (pentraxin-3, procalcitonin, adrenomedullin, lipopolysaccharide binding protein, interleukin-17A, lactate dehydrogenase, and C-creative protein), blood culture, antibiotic susceptibility test, and coagulation biomarkers (Prothrombin time, activated partial thromboplastin time, and international normalized ratio). Then, isolated Gram-negative bacteria were tested for extended-spectrum β-lactamase enzymes production by screening and combined disc tests.

Results: A total of 51.7% samples were blood culture positive for different Gram-negative bacteria, and *P. aeruginosa* (51.95%) was a more isolated bacterium. Both males and females were affected by sepsis in a ratio of 1.23:1 with different age groups. Extended-spectrum β-lactamase was estimated to be 77.2% by antibiotic profile, and the rate decreased using two double-disc synergy tests. This was confirmed by combined disc test at a rate of 41.35%. The most prevalent biomarkers were procalcitonin (88.16%), adrenomedullin (84.21%), pentraxin-3 (22.37%), and lipopolysaccharide binding protein (11.84%).

Conclusion: Sepsis is a life-threatening condition that can be diagnosed early by several blood biomarkers such as procalcitonin, adrenomedullin, and pentraxin-3 combined with a standard blood culture technique to improve the patient outcome.

Keywords: β-Lactamase, double-disc synergy test, SOFA score, sepsis biomarkers, Gram-negative bacteria

Introduction

Sepsis is a pathological syndrome initiated by a definite or suspected infection that leads to a high global mortality rate among people of all ages. Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection and "septic shock". The term "severe sepsis" was replaced by this new definition of sepsis.[1] The 2016 consensus definitions also recommend that the Sequential (Sepsis-related) Organ Failure Assessment (SOFA) criteria and "quick" (q)SOFA criteria to be used to identify sepsis, in place of the currently used systemic inflammatory response syndrome (SIRS) criteria, which was the basis for the previous definition of sepsis.[2] Sepsis results in a complex immune response characterized by pro-inflammatory and compensatory anti-inflammatory mechanisms. Therefore, most patients with sepsis rapidly exhibit signs of profound immunosuppression, with harmful consequences such as acute kidney injury and multi-organ failure caused by hospital-acquired Gram-negative bacilli or Gram-positive cocci, among immunocompromised patients and patients with chronic and debilitating diseases specifically ESBL producing *Enterobacterales*,

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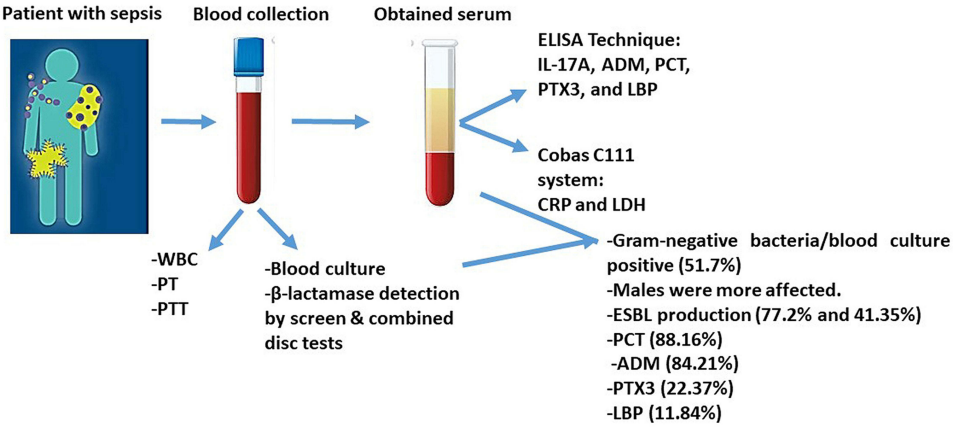
Graphical Abstract



multiple drug-resistant *Pseudomonas aeruginosa* (MDR-PA) with carbapenem-resistant *Enterobacterales*.[3,4] A study in the Rozh Halat Emergency Hospital, Erbil, Iraq, shows that sepsis is associated with high overall mortality rate (68%) and factors associated with high mortality were female gender, older age group, positive blood culture, wrong antibiotics therapy, less fluid resuscitation, multisource of infection, multi-organ failure, high lactic acid level, and high qSOFA score.[2]

The inflammatory response of sepsis starts by activation of the innate immune system, which is the primary immune response to microbial infection that produces a range of pro-inflammatory cytokines that trigger cytokines storm,[5] and these pro-inflammatory mediators and cytokines overproduction result in cell death in the form of apoptosis and necrosis.[6]

Several sepsis biomarkers had been commercialized over the past decade. They can promise the accurate detection of infection and prediction of the development of organ dysfunction and guide to antibiotic therapy.[7,8] In this respect, procalcitonin (PCT) is used as an indicator for antibiotic treatment as its level is higher in bacterial infections than in viral infections. Early detection of PCT in sepsis had been recommended to be associated with an unfavorable prognosis.[9,10] The pentraxin (PTX) family plays an important role in regulating inflammation, an acute phase protein, and it is a novel early diagnostic/prognostic biomarker in patients with sepsis.[11]

Another critical hormone involved in regulating the endothelium barrier and vascular tone is adrenomedullin (ADM), which associates with improvements in organ dysfunction scores and lowers mortality.[12,13] A novel predictor of sepsis progression and an attractive therapeutic target is interleukin-17A (IL-17A) which confers powerful protective effects against various infectious agents.[14] Moreover, lipopolysaccharide-binding protein (LBP) is widely reported as a biomarker to differentiate infected from non-infected patients; however, its diagnostic use for sepsis remains a matter of debate.[15] Thus, this study aimed to investigate inflammatory biomarkers in patients with sepsis and compare their role in the early detection of sepsis in Sulaimaniyah, Iraq.

Patients and Methods

Sample Size and Study Setting

This cross-sectional study was conducted on 147 patients suspected to have sepsis and admitted to the ICU and Dialysis Unit in Shar Teaching Hospital, Hiwa Hospital, Burn and Plastic Surgery Hospital, and Anwar Shexa Medical City in Sulaimaniyah, Iraq, from the beginning of the May to the end of December 2021. This study was conducted in accordance with the Declaration of Helsinki and signed informed consent was obtained from all patients before enrollment. Patients with suspected sepsis were allocated and diagnosed based on SOFA. Patients with two or more

SOFA scores considered to have sepsis and these scores include several variables (alteration of partial arterial oxygen pressure (PaO_2)/fraction of inspired oxygen (FiO_2) ratio, worsening thrombocytopenia, high total bilirubin level, hypotension with/without the use of vasopressors, increasing serum creatinine and declining urinary output and deteriorating, mean arterial pressure of ≤70 mmHg, and alteration in the level of consciousness by Glasgow Coma Scale, which recommended to help in sepsis diagnosis and prognosis.[16]

Inclusion Criteria

All patients confirmed to have sepsis with gram negative bacterial infection (adults and elderly) were enrolled in this study regardless of gender, ethnicity, and nationality.

Exclusion Criteria

Only pediatric age group had been excluded from this study.

Questionnaire

A well-designed questionnaire was used to collect the patient's sociodemographic data, including age, sex, and occupation, together with hospitalization duration and antibiotic profile history.

Study Protocol

An expert phlebotomist obtained about 10 mL of blood from each patient. First, a part of the blood (3.0 mL) was used to determine white blood cell (WBC) using the Medonic Beckman Coulter system (Boule, Sweden) and coagulation biomarkers such as prothrombin time (PT) and partial thromboplastin time (PTT) using Solea (Biolab, France). Then, INR (international normalized ratio) was calculated. Another part of the blood (7.0 mL) was used for serum collection after centrifugation at 3000 rpm for 15 minutes, aliquoted, and frozen at −80°C. Consequently, serum was used to estimate inflammatory biomarkers (CRP, PCT, ILT-17A, PTX3, ADM, LBP, and LDH).

Blood Culture

Two sets of blood cultures (10 mL of blood per blood culture bottle) were obtained before the initiation of antimicrobial therapy by locating of the peripheral venous site before skin antisepsis which achieved by using 2% chlorhexidine in 70% isopropyl alcohol. For this purpose, blood samples were inoculated directly into blood culture bottles specific for BacT/Alert (3D) automated blood culture system (Marcyl'Étoile, France) and processed based on the standard guidelines of the manufacturer. Then, bottles with positive signals were subjected to routine Gram-stain and further sub-cultured on different culture media called MaCconkey agar, Blood agar, and Chocolate agar (Oxoid Ltd., Basingstoke, United Kingdom) and incubated at 37°C for 18–24 hours. After incubation, the colony was characterized by colony appearance, Gram stain, and biochemical tests.[17]

Screen Test 1 (Antimicrobial Susceptibility Test; AST)

Bacterial isolates were identified using VITEK 2 compact automated system (bioMérieux, France) using Gram-Negative Identity Card (GN ID card) and VITEK 2 AST card except for colistins, cefepime, ceftazidime–avibactam (CZA), ceftriaxone, aztreonam, and amoxiclav, that were processed manually on Muller Hinton agar (MHA) plates using Kirby–Bauer disc diffusion method according to CLSI 2020 guideline.[18] In addition, standard bacterial strains of *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 25853 were used as quality control.

Screening for Potential ESBL-Producing Isolate (Screen Test-1)

Isolated Gram-negative bacteria were tested for ESBL enzyme production by screen and confirmatory (combined disc; CD test) methods. The isolates that showed an inhibition zone of ≤22 mm with ceftazidime (30 μg), ≤27 mm with cefotaxime (30 μg), ≤25 mm with ceftriaxone (30 μg), and ≤27 mm with aztreonam (30 μg) were considered potential ESBL enzyme producers. On the other hand, combined disc of ceftazidime–clavulanate (30/10 μg) was put 20 mm apart from ceftazidime disc alone (30 μg). Positive results were considered as an increase in the inhibition halo of the

combined disc (≥5 mm) compared with the ceftazidime disc alone.[19] *Klebsiella pneumoniae* ATCC 700603 was used as ESBL-positive control strain.[20]

Screen Test 2 (Double-Disc Synergy Test 1; DDST-1)

The ESBL production in the selected pathogens was identified by placing amoxicillin-clavulanic acid (20/10 µg), and 30 µg disc of each third-generation cephalosporin (ceftazidime, cefotaxime, ceftriaxone, and cefixime) at a distance of 20 mm from center to center to amoxicillin–clavulanic acid on MHA plates stroked with the tested organism. Development of the inhibition (keyhole phenomenon) towards the clavulanate disc indicated a potential ESBL positive[21] (Figure 1).

Screen Test 3 (Double-Disc Synergy Test 2; DDST-2)

DDST-2 was used for the first time to screen ESBL enzyme production using ceftazidime–avibactam (CZA) 30/20 µg at the center of 20 mm apart from 30 µg disc of each 3rd generation cephalosporins (ceftazidime, cefotaxime, ceftriaxone, and cefixime) on MHA plates stroked with the tested organism. The extension zone of inhibition (keyhole phenomenon) toward the inhibitor disc was recorded as a positive result (Figure 2).

Biomarker Estimation

Several biomarkers were assessed, including CRP, PTX3, IL-17A, lactate dehydrogenase (LDH), PCT, ADM and LBP. A commercial ELISA kit (Elabscience ELISA kit, China) was used to measure serum levels of IL-17A, ADM, PCT, PTX3, and LBP. In contrast, CRP and LDH were measured using an automated multipara metric analyzer Cobas C111 (Roche Diagnostics, Mannheim, Germany).

Statistical Analysis

The collected data were entered into the Statistical Package for the Social Sciences (SPSS, version 25.0, IBM Corporation, Armonk, NY, USA). The Chi-square test was used for the correlation between variables. Descriptive statistics were presented as mean ± standard deviation (SD) and as frequency/percentages for categorical variables. A P-value was set as very highly significant (P<0.000), highly significant (P<0.001), significant (P<0.05), and non-significant (P>0.05).

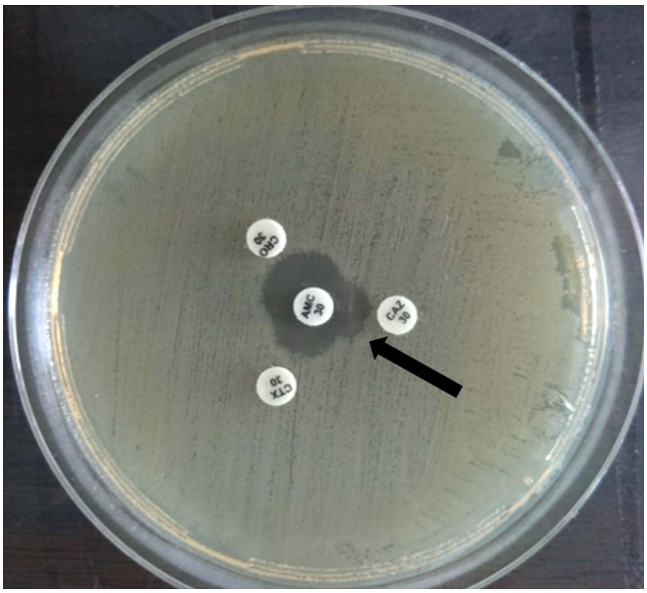


Figure 1 Double-disc synergy test 1. Positive test: Enhancement of zone of inhibition from amoxicillin–clavulanic acid towards ceftazidime disc and ceftriaxone disc (black arrow).

Figure 2 Double-disc synergy test 2. Positive test: Enhancement of zone inhibition from ceftazidime–avibactam towards ceftriaxone (black arrow).

Results

Out of 147 studied patients, 76 (51.7%) were positive blood cultures, while 71 (48.3%) were negative. All age groups were reported to have positive blood cultures with the highest value (47.37%) for >50 years; however, a significant correlation was found between age and blood culture results (P=0.001). The ratio of males to females affected by sepsis was 1.23:1 with no significant difference between age groups and blood culture results (P=0.3001). Regarding the patients' occupation, workers reported having more positive blood cultures (34.21%), followed by housewives (31.58%), while employers were the least (3.95%). In this regard, a positive correlation was found between patients' occupation and blood culture results (P=0.028). On the other hand, the hospitalization duration was 1 week for most patients (39.47%) with positive blood cultures; however, for blood culture negatives was >2-weeks in most patients (46.5%). No significant correlation was found between hospital stay duration in patients and their blood culture status (P=0.081) (Table 1).

Additionally, different clinical conditions were recorded as a source of infection to induce sepsis, including skin and soft tissue infection (42.18%), followed by respiratory tract infection (22.45%), then device-related infection (21.77%), bone and joint infection (9.52%), and chronic kidney disease (2.72%). At the same time, intra-abdominal infection was reported to be the least source of sepsis (1.36%). A significant relationship was found between sources of infection and sepsis rate (P=0.041) (Table 2).

Moreover, the most frequently isolated bacteria in this study were *Enterobacterales*, such as *Escherichia coli* (47.06%), followed by *Klebsiella pneumoniae* (41.18%), *Proteus mirabilis* (4.71%), *Raoultella ornithinolytica* (3.53%), *Enterobacter cloacae* (2.35%) and *Salmonella* (1.18%). Among the non-fermenter group, the most prevalent type was *Pseudomonas aeruginosa* (51.95%), followed by *Acinetobacter baumannii* (42.86%), *Burkholderia cepacia* (2.6%), then *Stenotrophomonas maltophilia* and *Alcaligenes faecalis* (1.3% each). A significant relationship was not found between Gram-negative bacterial isolates and using various screening tests (P>0.05) (Table 3).

Furthermore, we found the most resistant antibiotics to be third-generation cephalosporin at the rate of 72.5%, 70%, and 62.5% for cefotaxime, ceftazidime, and ceftriaxone, respectively, against *E. coli*. The resistance rate increased to 88.6% and 82.8% for ceftriaxone, cefotaxime, and ceftazidime against *K. pneumoniae*. The sensitivity patterns of *E. coli* and *K. pneumoniae* for colistins were 90% and 85.7%, respectively, followed by CZA (85% and 77.1%, respectively) and 4th generation cephalosporin (cefepime) (75% and 54.2%, respectively) (Supplementary Table 1).

In the non-fermenter group, *P. aeruginosa* showed resistance to at least four classes of antibiotics, and the most resistance was against amoxicillin–clavulanic acid (75%), followed by ceftriaxone (67.5%), ceftazidime (65%), and trimethoprim–sulfamethoxazole (52%). On the other hand, colistin was found to be the most effective drug (90%),

Table 1 Socio-Demographic Characteristics Among Patients with Sepsis

Socio Demographic Characteristic		Blood Culture		Total	P-value
		Positive	Negative		
		Frequency (%)			
Age (years)	>30	11 (14.47)	27 (38.0)	38 (25.85)	0.001*
	30–50	29 (38.16)	28 (39.4)	57 (38.78)	
	>50	36 (47.37)	16 (22.5)	52 (35.37)	
	Mean ± SD	49.46±18.31	39.08 ±17.68		
Sex	Male	45 (59.21)	36 (50.7)	81 (55.10)	0.3001
	Female	31 (40.79)	35 (49.3)	66 (44.90)	
Occupation	Housewife	24 (31.58)	31 (43.7)	55 (37.41)	0.028*
	Worker	26 (34.21)	21 (29.6)	47 (31.97)	
	Student	9.0 (11.84)	15 (21.1)	24 (16.33)	
	Retired	14 (18.42)	3.0 (4.2)	17 (11.56)	
	Employer	3.0 (3.95)	1.0 (1.4)	4 (2.72)	
Hospitalization duration (weeks)	1	30 (39.47)	16 (22.5)	46 (31.29)	0.081
	2	20 (26.32)	22 (31.0)	42 (28.57)	
	>2	26 (34.21)	33 (46.5)	59 (40.14)	
Total		76 (51.70)	71 (48.29)	147 (100)	

Notes: *Significant difference using Chi-square test, SD ± Standard deviation.

followed by CZA (80%) and cefepime/aztreonam (72%). In this regard, the resistance pattern was observed against most of the third-generation cephalosporins, quinolones, gentamicin and amikacin. In addition, carbapenem resistance was recorded specifically against *pseudomonas* and *Acinetobacter* (42–66%) (Supplementary Table 2).

In the current study, 162 isolates were screened for ESBL enzyme using three different screening methods. About 125 isolates (77.2%) were found to be ESBL producers among isolated Gram-negative bacteria. The rate of ESBL producers in the 2nd screen test (DDST-1) was 20.4%, and 69.8% in the 3rd screen test (DDST-2). However, the detection of ESBL was decreased to 41.35% among all isolates by CD test (P>0.05) (Table 4).

We tested several serum biomarkers and their correlation to the early detection of sepsis. PCT was detected among both culture positives and culture negatives at a rate of 88.16% and 74.6%, respectively (P=0.035), while LBP was 11.84% in positive blood cultures and 1.4% in negative blood cultures (P=0.012). Furthermore, ADM was raised among blood culture-positive (84.21%) and at nearly the same rate (88.7%) among culture-negative patients (P=0.424). IL-17A reported a non-significant correlation with a low rate of positivity (P=0.861), but PTX3 was found to be higher among culture-positive cases than negative cultures at a rate of 22.37% and 8.5%, respectively (P=0.02). LDH had an equal percentage among positive and negative culture groups (P=0.371).

Additionally, a higher rate (35.53%) for PT among positive blood cultures than negative blood cultures (19.7%) with a significant correlation (P=0.033), while insignificant differences for both PPT and INR in positive blood cultures compared to negative blood cultures (P>0.05) was reported. Finally, WBC showed higher account in positive blood cultures than negative blood cultures (21.05% vs 2.8%, respectively) with a significant correlation between them (P=0.001) (Table 5), also ROC curve has been calculated for sensitivity and specificity of the biomarkers (Figure 3). Moreover; both mean±SD and *t*-test was performed for each biomarker with their P-value (Table 6).

Table 2 Causes of Infection Among Patients with Sepsis

Type of Infection	Causes of Infection	N (%)	Total, N (%)	P-value
Skin and soft tissue infection	Burn	32 (36.8)	62 (42.18)	0.041*
	Postoperative wound	16 (43.2)		
	Abscess	14 (60.9)		
Respiratory tract infection	COVID-19	25 (28.7)	33 (22.45)	
	Aspiration pneumonia	8 (21.6)		
Device-related infection	Mechanical ventilation	18 (20.7)	32 (21.77)	
	Endotracheal tube	5 (13.5)		
	Catheters & C.V line	9 (39.1)		
Bone and joint infection	Road traffic accident	9 (10.3)	14 (9.52)	
	Falling from height	5 (13.5)		
Chronic kidney disease	Dialysis	2 (2.3)	4 (2.72)	
	Renal transplantation	2 (5.4)		
Intra-abdominal infection	Colostomy	1 (1.1)	2 (1.36)	
	Acute abdomen	1 (2.7)		
Total			147 (100)	

Notes: *Significant difference using Chi-square test.
Abbreviations: C.V line, central venous line; COVID-19, coronavirus disease-19.

Table 3 Isolated Gram-Negative Bacteria from Patients with Sepsis

Types of Gram-Negative Bacteria		Blood Culture Positive	Other Sources	Total	P-value
		N (%)			
Enterobacteriaceae	*Escherichia coli*	25 (51.02)	15 (41.67)	40 (47.06)	0.412
	Klebsiella pneumonia	17 (34.69)	18 (50)	35 (41.18)	
	Proteus mirabilis	3 (6.12)	1 (2.78)	4 (4.71)	
	Raoultella ornithinolytica	1 (2.04)	2 (5.56)	3 (3.53)	
	Enterobacter cloacae	2 (4.08)	0 (0.0)	2 (2.35)	
	Salmonella	1 (2.04)	0 (0.0)	1 (1.18)	
	Total	49 (30.24)	36 (22.22)	85 (100.0)	
Non-fermenter	*Pseudomonas aeruginosa*	21 (60)	19 (45.24)	40 (51.95)	0.202
	Acinetobacter baumannii	12 (34.29)	21 (50.0)	33 (42.86)	
	Burkholderia cepacia	0 (0.0)	2 (4.76)	2 (2.6)	
	Alcaligenes faecalis	1(2.86)	0 (0.0)	1 (1.3)	
	Stenotrophomonas maltophilia	1 (2.86)	0 (0.0)	1 (1.3)	
	Total	35 (21.6)	42 (25.92)	77 (100.0)	

Table 4 Number and Percentage of Phenotypic Tests for ESBL Detection

Gram-Negative Bacteria		Total	Screen Tests			Confirmatory Test (CD)	P-value
			Screen 1	Screen 2	Screen 3		
		N (%)					
Enterobacteriaceae	*K. pneumonia*	35(41.18)	32(46.38)	12(54.55)	31(47.69)	17(50)	0.436
	E. coli	40(47.06)	29(42.03)	8.0(36.36)	27(41.54)	14(41.18)	
	Proteus mirabilis	4.0(4.71)	4.0(5.8)	2.0(9.09)	4.0(6.15)	1.0(2.94)	
	Raoultella ornithinolytica	3.0(3.53)	3.0(4.35)	0.0(0.0)	3.0(4.62)	2.0(5.88)	
	Salmonella	1.0(1.18)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	
	Enterobacter cloacae	2.0(2.35)	1.0(1.45)	0.0(0.0)	0.0(0.0)	0.0(0.0)	
	Total	85(52.5)	69(42.6)	22(13.6)	65(40.1)	34(21)	
Non-fermenter	*P. aeruginosa*	40(51.95)	29(51.79)	3.0(27.27)	25(52.08)	17(51.52)	0.751
	Acinetobacter baumannii	33(42.86)	24(42.86)	8.0(72.73)	21(43.75)	14(42.42)	
	Stenotrophomonas maltophilia	1.0(1.3)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	
	Alcaligenes faecalis	1.0(1.3)	1.0(1.79)	0.0(0.0)	1.0(2.08)	0.0(0.0)	
	Burkholderia cepacia	2.0(2.6)	2.0(3.57)	0.0(0.0)	1.0(2.08)	2.0(6.06)	
	Total	77(47.5)	56(34.6)	11(6.8)	48(29.6)	33(20.4)	

Notes: Screen 1: Antibiotic profile, Screen 2: DDST1 (double-disc synergy test 1 by amoxicillin–clavulanic acid), Screen 3: DDST2 (double-disc synergy test2 by ceftazidime–avibactam), CD: combined disc test by (ceftazidime/ceftazidime–clavulanic acid).

Table 5 Sensitivity and Specificity of Inflammatory and Coagulation Biomarkers Among Patients with Sepsis

Inflammatory and Coagulation Markers		Blood Culture				Sensitivity%	Specificity%	AUC	Lower±Upper	P-value
		Negative		Positive						
		Fr	%	Fr	%					
PTX-3	Negative	65	91.5	59	77.63	91.5	22.4	0.571	0.478±0.664	0.02
	Positive	6	8.5	17	22.37					
PCT	Negative	18	25.4	9	11.84	25.4	88.2	0.573	0.48±0.667	0.035
	Positive	53	74.6	67	88.16					
ADM	Negative	8	11.3	12	15.79	11.3	84.2	0.476	0.383±0.57	0.424
	Positive	63	88.7	64	84.21					
LBP	Negative	70	98.6	67	88.16	98.6	11.8	0.553	0.46±0.646	0.012
	Positive	1	1.4	9	11.84					
IL-17A	Negative	70	98.6	75	98.68	98.6	01.3	0.5	0.406±0.594	0.861
	Positive	1	1.4	1	1.32					

(*Continued*)

Table 5 (Continued).

Inflammatory and Coagulation Markers		Blood Culture				Sensitivity%	Specificity%	AUC	Lower±Upper	P-value
		Negative		Positive						
		Fr	%	Fr	%					
LDH	Negative	37	52.1	34	44.74	52.1	55.3	0.541	0.447±0.634	0.371
	Positive	34	47.9	42	55.26					
CRP	Negative	6	8.5	1	1.3	0.08	0.99	0.304	0.131±0.477	0.042
	Positive	65	91.5	75	98.7					
PT	Negative	57	80.3	49	64.47	80.3	35.5	0.581	0.489±0.674	0.033
	Positive	14	19.7	27	35.53					
PTT	Negative	63	88.7	67	88.16	88.7	11.8	0.504	0.41±0.598	0.913
	Positive	8	11.3	9	11.84					
INR	Negative	55	77.5	60	78.95	77.5	21.1	0.494	0.40±0.588	0.828
	Positive	16	22.5	16	21.05					
WBC	Low	2	2.8	16	21.05	0.028	0.211	0.459	0.365±0.553	0.001*
	Normal	28	39.4	17	22.37	0.394	0224			
	High	41	57.7	43	56.58	0.577	0.566			
Total		71	100	76	100					

Notes: *Significant difference using Chi-square test. The cut-off value of PCT: 194.350–276.252 pg/mL, PTX-3: 1111–2987ng/mL, ADM: 3.888–6.416 ng/L, IL_17A: 46.507–83.576 ng/L, LBP: 4.467–12.715 ng/mL, LDH: 135–225U/L, CRP: 0–5 mg/L.

Abbreviations: Fr, frequency; AUC, area under curve; PTX-3, pentraxin-3; PCT, procalcitonin; ADM, adrenomedullin; LBP, lipopolysaccharide binding protein; IL-17A, interleukin-17A; LDH, lactate dehydrogenase; CRP, C-creative protein; PT, prothrombin time; PTT, partial thromboplastin time; WBC, white blood cell; INR, international normalized ratio.

Discussion

Early identification of infection severity and organ dysfunction is crucial in improving outcomes of patients with sepsis, as sepsis is a life-threatening and pathological syndrome mainly caused by a dysregulated host response to infection.[22] Thus, we aimed to find the blood biomarkers that are directly related to the early stage of sepsis development.

In the current study, males were more affected by sepsis than females (1.23:1). These findings were agreed with that found by Liu et al[1] and Xu et al[23] in China with Te Marvelde et al in Australia[24] who mentioned that most of the patients with sepsis were males. On the contrary, Xue et al in China found that most patients diagnosed with sepsis were females.[8] Also, we found the highest value of sepsis in patients aged >50 years, which is inconsistent with that found by Te Marvelde et al in Australia,[24] who found the highest sepsis rate in young patients with cancer and Lewis et al in sub-Saharan Africa who saw most patients with HIV infection aged 27–39 years.[25] However, these outcomes agreed with that observed by Liu et al in China (age range, 54–82 years)[1] and Luhr et al in Sweden (mean age= 62.7±4.2 years).[22] Regarding the hospitalization stay for patients, most patients (39.47%) with positive blood cultures stayed for 1 week; while most patients (46.5%) with blood culture negatives was stayed for >2-weeks. In this respect, Xu et al stated the median length of hospitalization and ICU stay for male patients to be higher (19.54 and 7.54 days) than female patients (16.49 and 6.75 days) ($P<0.001$ and $P=0.002$, respectively).[23]

On the other hand, we found that skin and soft tissue infection was the common cause of sepsis, followed by respiratory tract infection. In contrast, intra-abdominal infection was the least source of sepsis. These findings disagreed with that of Liu et al in China, who reported that soft tissue infection is the least cause of sepsis and

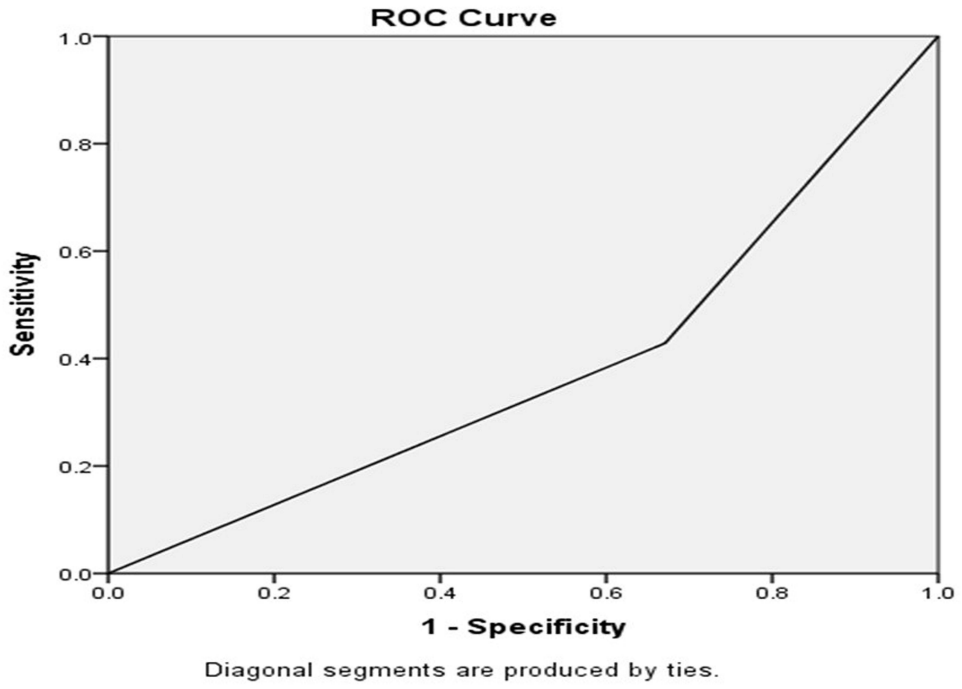


Figure 3 ROC curve of biomarkers.

pneumonia, followed by kidney diseases as the leading causes of sepsis.[1] Moreover, Xue et al found the most common cause of infection in sepsis to be pneumonia, followed by urinary tract infection, liver abscess, abdominal infection, and soft tissue infection.[8]

Table 6 Comparison of Mean Score of Different Biomarkers with Blood Culture Results Among Patients with Sepsis

Inflammatory and Coagulation Biomarkers	Blood Culture				Significant Test	
	Positive		Negative		*t*-test	P-value
	Mean	S.D	Mean	SD		
PTX-3	2722.8003	3499.83526	1822.1732	787.89482	2.118	0.036
PCT	1137.0573	622.74616	877.7539	739.97145	2.304	0.023
ADM	175.4357	200.36036	184.2598	183.78401	−0.278	0.782
LBP	0.2633	8.44317	−2.0932	4.06451	2.132	0.035
IL_17A	16.6605	28.55305	14.2690	20.23767	0.582	0.561
LDH	334.0658	327.73072	365.6338	563.56808	−0.418	0.676
CRP	358.4226	1805.86456	592.6276	2619.33682	−0.635	0.527
PT	15.9142	2.99991	14.9615	1.97646	2.257	0.026
PTT	31.4434	9.06557	32.1310	9.40893	−0.451	0.853
INR	1.5479	1.34983	1.5145	1.40893	0.147	0.884
WBC	11.7125	7.41576	14.3686	7.73359	−2.126	0.035

Abbreviations: SD, standard deviation; PTX-3, pentraxin-3; PCT, procalcitonin; ADM, adrenomedullin; LBP, lipopolysaccharide binding protein; IL-17A, interleukin-17A; LDH, lactate dehydrogenase; CRP, C-creative protein; PT, prothrombin time; PTT, partial thromboplastin time; WBC, white blood cell; INR, international normalized ratio.

Additionally, we found that most (51.7%) patients' blood samples were culture-positive (Gram-negative bacteria), which agreed with Xue et al, who reported Gram-negative bacteria (54.9%) as the main bacteria in sepsis.[8] Among isolated Gram-negative bacteria, *P. aeruginosa* (51.95%) was the most profound bacteria. This outcome agreed with those found by Aljanaby and Aljanaby in Iraq[26] and Emami et al in Iran,[27] who found *P. aeruginosa* as the most frequent pathogen 27.6% and 49.9%, respectively in burned patients with sepsis.

In this study, the prevalence rate of ESBL enzyme producers was recorded through an antibiotic profile in which isolates of the family *Enterobacterales* were produced ESBL enzyme at the highest level of than non-fermenter group (42.6%), especially *Klebsiella pneumonia* (46.38%) and *P. aeruginosa* (51.79%). In this regard, *P. aeruginosa* was found to be the most common ESBL-producer (35.9%) among septic patients in Iraq,[26] while Mansouri et al in Iran found ESBL-producer by 59.4% of *P. aeruginosa* isolates.[28] Thus, bacterial resistance is mostly observed against amoxicillin-clavulanic acid, followed by third-generation cephalosporin (cefotaxime, ceftazidime, and ceftriaxone) and then imipenem. These outcomes might be related to excessive use of these antibiotics by populations that cause selective pressure and the emergence of plasmid-mediated mutation of antibiotic resistance gene.[29,30] This is a worrying signal in low-income countries and is considered a major public health problem due to the limited laboratory services and therapeutic options despite inadequate infection control measures, especially in the healthcare setting.[31]

Infections caused by MDR Gram-negative organisms resulted in prolonged hospital stay with increased mortality and cost of management. Thus, new antimicrobial agents such as CZA were developed, combining the third-generation cephalosporin (ceftazidime) and non-β-lactam-β-lactamase inhibitor (avibactam). The effectiveness and safety of CZA globally have been demonstrated in managing MDR Gram-negative infections, including carbapenem-resistant *Enterobacterales*.[32] Due to the presence of CZA in medicine, most manufacturers use this antibiotic as a disc for susceptibility testing. In this study, the disc of CZA was used in AST and as a first trial in DDST-2 for ESBL as an inhibitor disc with a high rate of positive results compared to classical DDST-1. Also, no reported data supports this result and it is not even mentioned in CLSI or EUCAST.

During the last decade, the potential effort had been directed toward the identification and usefulness of biomarkers for early detection of sepsis that can help clinicians to predict and distinguish infection from host response to inflammation.[33] Accordingly, we found the highest expression level for CRP, and PCT, followed by ADM, LDH, PTX-3, LBP, and then IL-17A. In this regard, Zhang et al in China reported that IL-10, IL-17, and PCT biomarkers had a high diagnostic value for sepsis patients, particularly those admitted to the ICU.[34] In contrast, Piccioni et al in Italy concluded that ADM biomarker detection directly in the emergency department could contribute to improving the prognostic assessment of patients with sepsis.[35] Also, Wang et al in China reported the upregulation of the CRP and IL-17A in sepsis patients compared with those in healthy individuals,[36] while Vijayan et al in India suggested PCT as a promising diagnostic marker for sepsis[37] which increases in the presence IL-1, IL-6, or tumour necrosis factor α (TNF-α), probably due to the inhibition of PCT proteolysis.[38,39] Furthermore, Tian et al in China suggested that PTX-3 might be an early predictor to evaluate the severity of sepsis,[40] and Youness and Nahla in Egypt mentioned that LDH is a valuable biomarker in predicting sepsis in critically ill pediatric patients, especially when combined with predictive scoring systems.[41]

Moreover, LBP plays an essential role in innate immunity mechanisms, as it binds to the amphipathic lipid A of bacterial lipopolysaccharide (LPS), and transfers LPS to CD14 protein. By facilitating binding to the CD14 cell membrane molecules, LBP enhances the sensitivity of macrophages and other cells.[6] In this study, LBP had a weak expression in septic patients, which is similar to the findings of Chen et al's meta-analysis study in Taiwan, who reported a weak sensitivity and specificity of LBP in the detection of sepsis and they suggested that LBP was not recommended for clinical utilization as a single biomarker.[15]

Regarding the PT, PPT, INT, and WBC results, we observed a high level for each PT and WBC in culture-positive blood samples with no noticeable increase in the values of PPT and INR. These findings were also stated by[40] in China.

Conclusion

We concluded that sepsis is common among patients with pre-existing infection with 2 weeks of hospitalization. All age groups and both genders could get sepsis; however, elderly and male gender were predominance. Consequently, as a life-threatening condition, sepsis can be caused by various sources. Since most of our patients had a higher level of several

cytokines and developed endothelial cell injury in the initial phase of sepsis, thus it can be diagnosed by several biomarkers such as PCT, ADM, and PTX3 combined by standard culture techniques as these biomarkers may be helpful to evaluate severity and prognosis of sepsis in patients.

Abbreviations

ICU, intensive care unit; MDR, multidrug-resistant bacteria; SOFA, Sequential Organ Failure Assessment; WBC, white blood cell count; PT, prothrombin time; PTT, partial thromboplastin time; INR, international normalized ratio; LBP, lipopolysaccharide binding protein; CRP, C-reactive protein; PCT, procalcitonin; PTX3, pentraxin-3; ADM, aAdrenomedullin; IL-17A, interleukin-17A; LDH, lactate dehydrogenase; MHA, Muller–Hinton agar; CLSI, Clinical and Laboratory Standards Institute; ATCC, American Type Culture Collection; CD test, combined disc test; DDST, double-disc synergy test; COVID-19, coronavirus disease 2019; ESBL, extended-spectrum β-lactamase; ELISA, enzyme linked immunosorbent assay.

Data Sharing Statement

The data used to support the findings of this study are included within the article.

Ethical Approval and Consent to Participate

The protocol of this study was accepted with the approval of the Ethics Committee from the Directorate of Health in Sulaimani, Iraq, and the local medical ethics committee of the College of Medicine, University of Sulaimani (No. 78-UoS on May 18, 2021). The participants were enrolled in the study after being informed about the procedure and providing their written consent.

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Disclosure

The authors report no conflicts of interest in this work.

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