ORIGINAL ARTICLE

Soluble Programmed Cell Death Protein-1(sPD-1) as an Early Diagnostic and Prognostic Marker for Sepsis Following Respiratory Tract Infections

[1]Rasha A. Elsayed, [1]Ahmed S. Osman, [1]Aghadear A.H. Taha*, [2]Nashwa A. Amin, [1]Amira E. Ramadan

[1]Medical Microbiology and Immunology Department, Faculty of Medicine, Benha University, Egypt
[2]Chest and ICU Departments of Benha University Hospital

Key words:
Sepsis, sPD-1, ELISA, Bact Alert 3D system, K.pneumoniae, P.aeruginosa

Corresponding Author:
Aghadear Ahmed Hamed Taha
Department of Medical Microbiology and Immunology, Faculty of Medicine, Benha University, Egypt
Tel: 01069598692
aghadearahmedtaha45@gmail.com

ABSTRACT

Background*: Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection. Soluble Programmed cell death protein-1(sPD-1) increased in patients with sepsis and involved in immunosuppression. It is associated with a poor prognosis, severity and 28-day mortality.* ***Objectives:*** *Identification of the caustive agents of sepsis using Bact Alert 3D system, measurement of serum level of sPD-1 in patients with sepsis compared to control group by ELISA and evaluation of sPD-1 as a diagnostic and prognostic marker for the severity and 28-day mortality.* ***Methodology:*** *Our study was done on 40 patients diagnosed as sepsis in Chest and Intensive Care Units Departments of Benha University Hospital. Blood samples were withdrawn from the patients for blood culture, Subcultures and biochemical tests were done for identification of the caustive agents of sepsis and ELISA is used for for measurment of sPD-1 level in patients and control group.* ***Results:*** *According to the causative pathogens, Klebsiella pneumoniae was the most frequently isolated organism (32.5%), followed by Pseudomonas aeruginosa (22.5%) and Staphylococcus aureus (17.5%). According to sPD-1, the mean serum level was significantly higher in the case group (17.58 ± 15.05 pg/mL) compared to the control group (0.53 ± 0.37 pg/mL). SPD-1 levels were markedly higher in non-survivors compared to survivors.* ***Conclusion:*** *sepsis is a common disease with multimicrobial causes. Klebsiella pneumoniae is the main causative bacteria for sepsis. sPD-1 is a promising diagnostic marker for sepsis with high sensitivity (97.5%), and specificity (95.0%), and prognostic marker for the severity and 28-day mortality*

INTRODUCTION

According to the World health organization (WHO); Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection. Septic shock is a subset of sepsis in which underlying circulatory, cellular, and metabolic abnormalities contribute to a greater risk of mortality than that occurs by sepsis alone.[1]

Annual worldwide estimates include 31.5 million sepsis cases, 19.4 million severe sepsis cases and 5.3 million deaths due to sepsis.[2]

The pathogenesis of sepsis depends mainly on the host immune response. Any infection stimulates the immune system to elicit an inflammatory response. If dysregulated inflammation occurs, it will result in sepsis which comprise two phases: the first hyperinflammatory state (Cytokine storm) followed by a second phase of immune suppression (immune paralysis).[3]

This immunosuppression is caused by the excessive release of anti-inflammatory cytokines and the decline in the number and function of immune cells and continuous immunosuppression will lead to increased mortality. [4]

Recent studies have shown that Soluble Programmed cell death protein-1(sPD-1) also known as CD279 which is a member of the B7-CD28 immunoglobulin superfamily is increased in patients with sepsis and involved in immunosuppression in sepsis, it is associated with a poor prognosis and it shows valuable predictive ability for the severity and 28-day mortality of severe sepsis and septic shock during the first week of ICU treatment, So it is one of the most promising biomarkers of sepsis.[4]

The most common pathogen in the septic patients is *Escherichia coli* (21.5%), followed by *Klebsiella pneumoniae* (9.0%), Methicillin-Sensitive *Staphylococcus aureus* (*MSSA)* (6.5%), and *Streptococcus pneumonia* (5.0%).[5]

The dominant pathogens in patients with a respiratory source of infection are *Streptococcus*

pneumoniae and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*).[6]

Blood culture is an excellent method for diagnosis of sepsis. Automated blood culture system makes the processing of blood cultures more efficient. It eliminates the need for blind or terminal subcultures of bottles, reducing the number of times bottles must be handled during processing, speeding detection of microbial growth, maximizing blood culture sensitivity and specificity.[7] One of such system is the BacT/Alert 3D , an automated system for blood cultures. BacT/Alert system is used for detection of bloodstream infections with bacteria or yeasts.[8]

METHODOLOGY

This research was conducted between June 2024 to February 2025 at the Microbiology and Immunology Department, Faculty of Medicine, Benha University.

This cross sectional study was conducted on 40 adults clinically suspected with sepsis and admitted to Chest and ICU Departments at Benha University Hospital, and 20 apparently healthy control subjects with matched age and sex for the patient group.

Ethical consideration

The study protocol was explained to all subjects participated in this study and an informed written consent was taken from all the patients and control subjects. This study was approved by the Ethical Committee of Faculty of Medicine, Benha University, MS 18-2-2024.

Inclusion criteria:

- Patients aged ≥18 years with a sepsis diagnosis according to the Sepsis-3 criteria.
- Patients with sepsis who had an acute organ dysfunction with a total Sequential Organ Failure Assessment (SOFA) score of ≥2 points consequent to an infection.
- Patients with septic shock who had hypotension with a vasopressor requirement to maintain a mean arterial pressure (MAP) of ≥65 mmHg and lactate concentration of ≥2 mmol/L (18 mg/dL) in the absence of hypovolemia.

Exclusion criteria were as follows:

- Patients who had preexisting coagulopathy.
- Who had intake anticoagulant or antiplatelet medication.
- Who received blood transfusion within the last 3 months.
- Liver cirrhosis at stage C.
- End-stage chronic kidney disease with intermittent hemodialysis.
- Who were diagnosed with cancer.
- Idiopathic thrombocytopenic purpura (ITP), multiple myeloma, hemolytic uremic syndrome, which could affect function and counts of platelets.
- Human immunodeficiency virus (HIV) infection or viral hepatitis.
- Chemotherapy, or radiation therapy within the past six months.

All patients were subjected to: Laboratory investigations result as Complete blood count (CBC), C-reactive protein (CRP), International Normalized Ratio (INR), Prothrombin time (PT), Lactate, Total bilirubin, Troponin, Urea, Creatinine, Aspartate transaminase (AST), Blood culture, Sequential Organ Failure Assessment (SOFA) score were collected from patients' files.

Microbiological examination

- Under complete aseptic conditions blood samples will be taken for both patients and controls.10 ml of blood was collected from each patient clinically suspected of sepsis, 8 ml of blood was inoculated into BacT/ALERT PF Plus Culture Bottles (Automated culture bottles) at the bedside of patient.The other 2 ml blood was inoculated in serum blood collection tubes vacutainer for further ELIZA assay of sPD1 for all patients and controls.
- **Blood culture**: the inoculated BacT/ALERT bottles incubated in the BacT/ALERT®3D instrument bioMérieux until microbial growth is detected, then subcultures were done on suitable media (Blood agar medium, MacConkey's agar medium, Sabouraud's dextrose agar medium) (Oxoid UK). After 24 hours of incubation, Bacteria were identified by standard bacteriological methods.[9]
- **Measurement of Soluble programmed cell death protein-1:** sPD-1 levels were measured in the serum of patients and control groups by enzyme-linked immunosorbent assay.

RESULTS

The present study was conducted on 40 sepsis cases and 20 control .According to demographic data, in terms of age, there was no significant difference between the case and control groups, with mean values of 59.92 ± 14.61 years and 57.95±18.66 years, respectively (p=0.655). Regarding sex distribution, the proportions of males (52.5% vs. 55.0%) and females (47.5% vs. 45.0%) were comparable between groups, showing no statistical difference (p=1.000), as shown in table (1).

According to the causative pathogens, *Klebsiella pneumoniae* was the most frequently isolated organism (32.5%), followed by *Pseudomonas aeruginosa* (22.5%) and *Staphylococcus aureus* (17.5%). Regarding fungal infections, *Candida albicans* was detected in 12.5% of cases, while *Coagulase-negative Staphylococci* had a similar prevalence (12.5%). *Escherichia coli* was the least common isolate (2.5%) as shown in Figure (1).

Soluble programmed cell death protein-1 in the Study Groups is shown in table (2).

Table 1: Demographic data in Study Groups

Parameter	Category	Case group (n=40)	Control group (n=20)	Test Results
Age (years)	Mean ± SD	59.92 ± 14.61	57.95 ± 18.66	t: 0.449, p=0.655
	Median (Range)	63.50 (19.00-82.00)	56.00 (28.00-84.00)	
Sex	Male	21 (52.5%)	11 (55.0%)	X^2: 0.000, p=1.000
	Female	19 (47.5%)	9 (45.0%)	

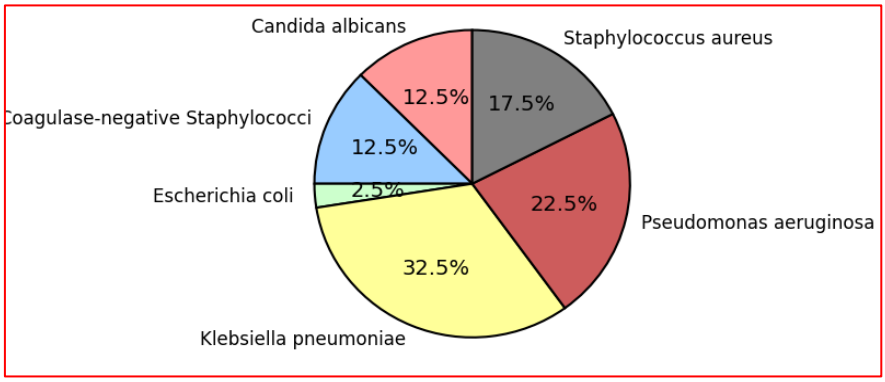

Fig. 1: Blood culture distribution in Case group

Table 2: Soluble programmed cell death protein-1 in Study Groups

Parameter	Category	Case group (n=40)	Control group (n=20)	Test Results
sPD-1 (pg/mL)	Mean ± SD	17.58 ± 15.05	0.53 ± 0.37	t: 5.047, p<0.001*
	Median (Range)	11.78 (1.53-55.57)	0.35 (0.24-1.38)	

sPD-1: Soluble Programmed Cell Death Protein-1, t: Student t test, * for significant p value (<0.05)

According to sPD-1 expression, the mean serum level was significantly higher in the case group (17.58 ± 15.05 pg/mL) compared to the control group (0.53 ± 0.37 pg/mL) (p<0.001).

Soluble programmed cell death protein-1 in cases survivors and non-survivors is shown in table (3).

Outcome in cases group is shown in table (4).

In terms of clinical course, mechanical ventilation was required in 75.0% of sepsis patients. Regarding survival, 72.5% of patients did not survive after the 28-day follow-up, while only 27.5% survived.

Validity of sPD-1 is shown in table (5).

Table 3: Soluble programmed cell death protein-1 according to outcome

Parameter	Category	Non survivor (n=29)	Survivor (n=11)	Test Results
sPD-1 (pg/mL)	Mean ± SD	22.95 ± 14.37	3.44 ± 1.16	t: 4.463, p<0.001*

sPD-1: Soluble Programmed Cell Death Protein-1, t: Student t test, * for significant p value (<0.05)
SPD-1 levels were markedly higher in non-survivors (22.95 ± 14.37 pg/mL) compared to survivors (3.44 ± 1.16 pg/mL) (p<0.001).

Table 4: Outcome in cases group

Parameter	Category	Case group (n=40)
On mechanical ventilation	n(%)	30 (75.0%)
Survivor after follow-up for 28 days	n(%)	11 (27.5%)
Non-survivor after follow-up for 28 days	n(%)	29 (72.5%)

Table 5: Validity of sPD-1

Variable	AUC	95% CI	p-value	Cut off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Sepsis prediction	0.975	0.921 - 1.000	<0.001*	1.5	97.5	95.0	97.5	95.0	96.7
Survival prediction	0.690	0.505 - 0.862	0.038*	11.0	69.0	72.7	87.0	47.1	70.0

sPD-1: Soluble Programmed Cell Death Protein-1, AUC: Area Under the Curve, CI: Confidence Interval, PPV: Positive Predictive Value, NPV: Negative Predictive Value.

DISCUSSION

Sepsis is still a major cause of morbidity and mortality worldwide, despite improvements in medical care and raised awareness.[10]

This work aimed to identify the causative agents of sepsis by blood culture using BacT/Alert 3D system, measurement of serum level of sPD-1 in patients with sepsis compared to control group by ELISA technique and evaluation of sPD-1 as diagnostic and prognostic marker for the severity and 28-day mortality during Sepsis.

Our study comprised 40 patients with sepsis , according to demographic data, in terms of age, the mean values were 59.92 ± 14.61 years.Regarding sex distribution, the proportions of males and females were (52.5% vs. 47.5%). The results agreed with Madkour et al.[11]; their study included 100 cases with sepsis out of 403 admitted cases in the same duration. Among sepsis patients, 72% were males and 28%were females, with mean age 51.62 ± 18.62 years.

In our study; According to the causative pathogens, *Klebsiella pneumoniae* was the most frequently isolated organism (32.5%), followed by *Pseudomonas aeruginosa* (22.5%) and *Staphylococcus aureus* (17.5%). Regarding fungal infections, *Candida albicans* was detected in 12.5% of cases, while Coagulase-negative Staphylococci had a similar prevalence (12.5%). *Escherichia coli* was the least common isolate (2.5%). The results were consistent with the findings of Madkour et al.[11] research who stated that the total number of patients with sepsis admitted at RICU during his study were 100 patients, the causative organisms of infection among sepsis cases were *Klebsiella pneumoniae* (29%), followed by *Pseudomonas aeruginosa* (19%), Acid fast bacilli (17%), *Acinetobacter* species (16%), *Candida* (14%), *Staphylococcus aureus* (10%), *Escherichia coli* (8%) , and *Proteus* species (2%) .

The results were also similar to that observed by Tabah et al.[12]; in his study the most frequent pathogens were Gram-negative bacteria (59.0%), predominantly Klebsiella spp. (27.9%), Acinetobacter spp. (20.3%), *Escherichia coli* (15.8%), and Pseudomonas spp. (14.3%).

In our study; according to sPD-1 expression, the mean serum level was significantly higher in the case group (17.58 ± 15.05 pg/mL) compared to the control group (0.53 ± 0.37 pg/mL) ($p<0.001$). The results agreed with ***Liu et al.,***[13] who conducted his study on 91 patients with sepsis and stated that his study suggested that serum sPD-1 levels were significantly increased in septic patients compared with Healthy controls (HC) ($P = 0.000$).

In this study; sPD-1 levels were markedly higher in non-survivors (22.95±14.37 pg/mL) compared to survivors (3.44±1.16 pg/mL) ($p<0.001$), and in terms of sepsis prediction, sPD-1 demonstrated excellent diagnostic performance with an AUC of 0.975 ($p<0.001$), a cutoff value of 1.5 pg/mL, high sensitivity (97.5%), and specificity (95.0%), leading to an overall accuracy of 96.7%. Regarding survival prediction, sPD-1 showed moderate prognostic value with an AUC of 0.690 ($p=0.038$), a cutoff of 11.0 pg/mL, sensitivity of 69.0%, specificity of 72.7%, and an accuracy of 70.0%. The results agreed with Zhao et al.,[14] who reported that, as the severity of sepsis increased, the level of sPD-1 gradually increased as well. The cutoff value of the level of sPD-1 (2.7 ng/mL) on day 7 for the prediction of the 28-day mortality showed the highest specificity (97.3%). A Kaplan–Meier survival analysis indicated that patients with sPD-1 levels higher than the cutoff values of 2.66 ng/mL on day 1 or 2.7 ng/mL on day 7 had a lower probability of survival at 28 days **.**

In the present study mechanical ventilation was required in 75.0% of sepsis patients .Regarding survival, 72.5% of patients did not survive after the 28-day follow-up, while only 27.5% survived. The results were similar to that observed by ***Mohamed et al.*** [15] who stated that (70%) of patients with severe sepsis underwent invasive mechanical ventilation. The mortality rate among these patients was found to be significantly higher (85.7%) as compared with those who were treated without invasive mechanical ventilation (25.0%).

CONCLUSION

Sepsis a common multimicrobial disease with *Klebsiella pneumoniae* is the main causative agent of sepsis following respiratory tract infections. sPD-1 is an excellent diagnostic marker for sepsis with high sensitivity (97.5%), and specificity (95.0%), and prognostic marker for the severity and 28-day mortality during Sepsis.

Declarations:

Consent for publication: Not applicable

Availability of data and material: Data are available upon request.

Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

This manuscript has not been previously published and is not under consideration in another journal.

Funding: Authors did not receive any grants from funding agencies.

REFERENCES

1. Guarino M, Perna B, Cesaro AE, Maritati M, Spampinato MD, Contini C, De Giorgio R. Update on Sepsis and Septic Shock in Adult Patients: Management in the Emergency Department. Journal of Clinical Medicine. (2023) 12(9):3188.
2. Gharamti AA, Samara O, Monzon A, Montalbano G, Scherger S, DeSanto K, Shapiro L. Proinflammatory cytokines levels in sepsis and healthy volunteers, and tumor necrosis factor-alpha associated sepsis mortality: A systematic review and meta-analysis. Cytokine. 2022; 158, 156006.
3. Sari MI, Ilyas S. The expression levels and concentrations of PD-1 and PD-L1 proteins in septic patients: a systematic review. Diagnostics, 2022; 12(8): 2004.
4. Zhong S, Yin Y. Regulatory role of the programmed cell death 1 signaling pathway in sepsis induced immunosuppression. Frontiers in Immunology. 2023; 14:1183542.
5. Umemura Y, Ogura H, Takuma K, Fujishima S, Abe T, Kushimoto S, Gando S. Current spectrum of causative pathogens in sepsis: a prospective nationwide cohort study in Japan. International Journal of Infectious Diseases. 2021; 103:343-351.
6. Eshwara VK, Mukhopadhyay C, Rello J. Community-acquired bacterial pneumonia in adults: An update. Indian Journal of Medical Research. 2020; 151(4):287-302.
7. Wilson ML, Weinstein MP, Reller LB. Automated blood culture systems. Clinics in laboratory medicine. 1994; 14(1):149-169.
8. Mirrett S, Hanson KE, Reller LB. Controlled clinical comparison of VersaTREK and BacT/ALERT blood culture systems. Journal of clinical microbiology, (2007) 45(2), 299-302.
9. Giuliano C, Patel CR, Kale-Pradhan PB. A guide to bacterial culture identification and results interpretation. Pharmacy and Therapeutics, 2019; 44(4): 192.
10. La Via L, Sangiorgio G, Stefani S, Marino A, Nunnari G, Cocuzza S, Maniaci A. The global burden of sepsis and septic shock. Epidemiologia. 2024; 5(3):456-478.
11. Madkour AM, ELMaraghy AA, Elsayed MM. Prevalence and outcome of sepsis in respiratory intensive care unit. The Egyptian Journal of Bronchology. 2022; 16(1):29.
12. Tabah A, Buetti N, Staiquly Q, Ruckly S, Akova M, Aslan AT, Timsit JF. Epidemiology and outcomes of hospital-acquired bloodstream infections in intensive care unit patients: the EUROBACT-2 international cohort study. Intensive care medicine. 2023; 49(2):178-190.
13. Liu M., Zhang X, Chen H, Wang G, Zhang J, Dong P, Wang L. Serum sPD-L1, upregulated in sepsis, may reflect disease severity and clinical outcomes in septic patients. Scandinavian Journal of Immunology. 2017; 85(1):66-72.
14. Zhao Y, Jia Y, Li C, Fang Y, Shao R. The risk stratification and prognostic evaluation of soluble programmed death-1 on patients with sepsis in emergency department. The American Journal of Emergency Medicine. 2018; 36(1);43-48.
15. Mohamed AKS, Mehta AA, James P. Predictors of mortality of severe sepsis among adult patients in the medical Intensive Care Unit. Lung India. 2017; 34(4):330-335.