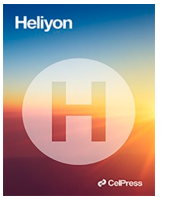
Heliyon 10 (2024) e28620

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Diagnostic and prognostic significance of apelin-13, APJ for sepsis in the emergency department: A prospective study

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ARTICLE INFO

Keywords:
Apelin-13
APJ
$CD4^+$ T cells
Sepsis
Emergency department

ABSTRACT

Objectives: This study aimed to assess the diagnostic, risk stratification, and prognostic capabilities of apelin-13 and APJ in comparison to procalcitonin (PCT) for septic patients presenting to the emergency department (ED).

Methods: Two hundred and thirty-eight patients meeting the Third International Consensus Definition (Sepsis-3) criteria were enrolled from Beijing Chaoyang Hospital's ED, along with a control group of forty healthy individuals. Patients were categorized into two groups based on disease severity: those with sepsis or septic shock. Plasma levels of apelin-13, $CD4^+$ Th cells, and PCT were measured. The expression levels of plasma APJ mRNA were quantified using real-time fluorescence quantitative PCR (RT-qPCR) methodology. The Sequential Organ Failure Assessment (SOFA) score was determined at the time of enrollment. The prognostic values of apelin-13 and APJ was evaluated in comparison to that of PCT and the SOFA score. All patients were followed up for a duration of 28 days.

Results: The plasma concentrations of apelin-13 and APJ exhibited a positive correlation with the severity of sepsis, while the number of $CD4^+$ T cells decreased in septic patients. The areas under the receiver operating characteristic (AUC) curves for apelin-13 and APJ in the diagnosis and prediction of 28-day mortality were greater than that of PCT. In non-survivors at the 28-day follow-up, the plasma levels of apelin-13 and APJ were significantly higher compared to survivors. Furthermore, apelin-13 levels were notably higher in cases of sepsis-induced cardiomyopathy (SICM) than in those without SICM. Apelin-13 and APJ emerged as independent predictors of 28-day mortality among septic patients.

Conclusions: Apelin-13 and APJ demonstrate value in the assessment of risk stratification, early diagnosis, and prognosis of sepsis in the ED. Apelin-13 also proves to be an effective biomarker for assessing the prognosis of SICM in the ED. Sepsis may lead to immune function suppression.

1. Introduction

Sepsis represents a critical medical condition marked by organ dysfunction ensuing from the systemic inflammatory response to infection. Despite considerable progress in intensive care and medical technology, sepsis continues to exhibit with a significant

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https://doi.org/10.1016/j.heliyon.2024.e28620
Received 27 September 2023; Received in revised form 12 March 2024; Accepted 21 March 2024
Available online 26 March 2024

mortality rate, particularly in the emergency department (ED) [1,2]. The development of sepsis is an intricately woven process influenced by the delicate equilibrium between microbial virulence and host immunity [3]. Early diagnosis, accurate classification, and timely intervention in the early phases of sepsis are pivotal in reducing mortality. Nevertheless, the current sepsis diagnostic framework hinges upon a series of laboratory analyses, biological assessments, and microbiological examinations, potentially leading to treatment delays [4]. The quest for novel, highly sensitive indicators to facilitate early detection, stratification of risk, and evaluation of prognosis in sepsis is of paramount importance in the pursuit of decreased mortality rates.

Apelin, a peptide hormone recently uncovered, has been identified as the native ligand for the formerly unassigned receptor APJ [5], first recognized by O'Dowd et al. [6]. Initially secreted as a prepropeptide comprising 77 amino acid residues, apelin undergoes cleavage to generate various active forms, among which apelin-13 has been recognized as the primary isoform present in human plasma [7]. The apelin/APJ signaling pathway exerts influence across a diverse range of physiological realms, including inflammatory reactions, neurological functions, metabolic processes, hypertension regulation, respiratory functions, gastrointestinal activities, hepatic functions, renal activities, and the pathogenesis of various cancers [8–10]. Several clinical investigations have substantiated that serum apelin-13 levels are elevated in septic patients, signifying its diagnostic value in the context of sepsis [11]. Furthermore, as sepsis frequently coincides with cardiomyocyte injury, animal studies have indicated that exogenous administration of apelin may mitigate inflammation, apoptosis, and augment autophagy in lipopolysaccharide-induced cardiac dysfunction [12,13]. Moreover, apelin-13 demonstrates the capacity to alleviate inflammatory responses induced by lipopolysaccharide and attenuate acute lung injury [14,15].

However, prior studies, characterized by limited sample sizes, failed to fully elucidate the predictive potential of apelin and APJ in sepsis patients [11]. In this study, we sought to ascertain whether plasma levels of apelin, APJ, and immune status exhibit disparities according to the severity of sepsis. Our objective was to assess their effectiveness for early diagnosis and prognostic evaluation of sepsis, in comparison to traditional infection biomarkers, with the overarching goal of identifying dependable, prospective diagnostic and preventative biomarkers.

2. Materials and methods

2.1. Study population

An observational study was conducted in the emergency departments (EDs) of two separate hospitals, Beijing Chaoyang Hospital and Beijing Shijitan Hospital, with annual ED admission rates of approximately 250,000 and 100,000, respectively. Between August 2019 and December 2022, patients consecutively admitted to the ED and diagnosed with sepsis or septic shock based on the criteria outlined in the Third International Consensus Definition (Sepsis-3) were considered eligible for inclusion [16]. To be eligible for participation, individuals had to be at least 18 years old and express a willingness to take part in the study. Exclusion criteria comprised: 1) individuals with an immunocompromised status, 2) individuals with a history of prolonged corticosteroid or immunosuppressive drug usage, and 3) individuals diagnosed with acute myocardial infarction or chronic cardiac insufficiency. A control group comprising healthy individuals was recruited from the physical examination centers. Blood samples were obtained from these individuals upon admission to the centers on the same day. We enrolled a cohort comprising 238 patients, who were monitored for a duration of 28 days or until the event of mortality. Septic patients were separated into two categories: those with septic shock and those without, and compared to the healthy control group. Mainstay therapy for sepsis patients included antibiotic administration and fluid resuscitation. Furthermore, additional interventions such as the use of vasoactive agents and mechanical ventilation are implemented based on clinical judgment and patient condition.

2.2. Clinical data and sample collection

Upon enrollment, demographic information such as the patient's name, age, sex, medical history, and vital signs was promptly recorded. Relevant laboratory examinations were performed and documented within 24 h. Sequential Organ Failure Assessment (SOFA) scores were determined using clinical parameters including the oxygenation index, platelet count, bilirubin levels, mean arterial pressure, administration of vasoactive drugs, Glasgow Coma Scale score, creatinine levels, and urine output. Upon admission to the ED, venous blood samples were drawn into tubes containing either heparin or ethylenediamine tetraacetate (EDTA). Subsequently, the samples were centrifuged at 3000 rpm for 5 min and then stored at $-80\ ^{\circ}C$ until they were ready for assay.

The levels of apelin-13 and APJ were assessed using commercially available kits (Art.No.EK12655 and EK4948, Nanjing Chuanbo Biotech Co., CHINA) employing enzyme-linked immunosorbent assay (ELISA) and real-time fluorescence quantitative PCR (RT-qPCR) techniques. SOFA scores were calculated upon ED admission. Patients with sepsis were categorized into two groups based on their 28-day survival outcome: survivors, who were defined as those who remained alive throughout the follow-up period, and non-survivors, who succumbed to their condition or related complications. In addition, septic patients underwent monitoring for the occurrence of septic myocardial injury, characterized by a reduction in ejection fraction (EF) to less than 50% and a decrease of at least 10% compared to their baseline EF, with subsequent recovery observed within a period of 2 weeks [17]. In cases where the baseline EF was not available, it was determined as an increase of $>10\%$ compared to the initial EF measured upon admission. Furthermore, no inotropic agents, including dobutamine and epinephrine, were utilized prior to the assessment with transthoracic echocardiography.

2.3. Statistical analysis

All statistical analyses were conducted using IBM SPSS Statistics 25.0 and GraphPad Prism 9.0. Descriptive statistics, including the

median and interquartile range for non-normally distributed data or the mean and standard deviation for normally distributed data, were computed. Group differences were evaluated using t-tests and one-way ANOVA analysis. The results of secondary analyses remain inconclusive. To predict 28-day mortality in sepsis, receiver operating characteristic (ROC) curves were constructed. The area under the curve (AUC) was determined, along with prognostic parameters such as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), calculated based on optimal thresholds identified through ROC curve analysis. AUC comparisons were performed using the Z-test formula. Multivariable logistic regression analysis was employed to identify the independent factors associated with 28-day mortality. Choose the infection-related indicators as the included variables. The predictive significance of 28-day survival was evaluated using the Kaplan-Meier estimator and Cox regression hazards analysis. All statistical analyses were conducted with a two-tailed approach, considering p-values less than 0.05 as statistically significant.

3. Results

3.1. Clinical data characteristics and baseline data

Out of the 238 patients included in the study, 66 were diagnosed with septic shock while 172 were classified as part of the septic group, categorized by disease severity. In terms of age and sex distribution, no significant differences were observed among the three groups of enrolled subjects, namely the sepsis group, septic shock group, and control group (Table 1). Table 1 displays the baseline characteristics, diseases, and associated infections observed in the participants included in our study. Analysis revealed a 28-day mortality rate of 21.5% among individuals diagnosed with sepsis, whereas for those in septic shock, the mortality rate was notably higher at 53.0%. Importantly, statistical analysis confirmed significant differences between these two groups ($p < 0.01$).

3.2. Levels of Apelin-13, APJ, apelin-13/APJ ratio, and $CD4^+$ T cells in enrolled subjects

Table 1 displays the average values of apelin-13, APJ, apelin-13/APJ ratio, PCT, CRP, IgG, $CD4^+$ T cells, lactate, CTNI, BNP, EF and SOFA scores in each group. Apelin-13(Fig. 1A), APJ(Fig. 1B), $CD4^+$ T cell(Fig. 1C), PCT and lactate at ED admission differed significantly across the various groups. In comparison to the group of healthy controls, levels of apelin-13, APJ, PCT, CRP, and lactate demonstrated a statistically significant increase among sepsis patients ($p < 0.01$), while $CD4^+$ T cell counts exhibited a notable decrease ($p < 0.01$). Furthermore, apelin-13, APJ, PCT, lactate, CTNI, and SOFA scores exhibited statistically significant elevation in septic shock compared to sepsis ($p < 0.01$), accompanied by a concurrent decrease in $CD4^+$ T cell count ($p < 0.01$). However, apelin-

Table 1
Patient characteristics in control and sepsis groups.

	Controls	All patients		P value
		sepsis	septic shock	
Number	40	172	66	–
Age(years)	74.17 ± 1.75	71.69 ± 1.03	73.15 ± 1.39	0.09
Male, n (%)	19(48)	89(52)	35(53)	0.85
WBC(× 10^9/L)	5.57 ± 0.22	13.84 ± 0.57	15.01 ± 1.04	< 0.01
apelin-13(pg/ml)	505.69 ± 51.97	1290.30 ± 44.91	2324.63 ± 110.14	< 0.01
APJ(pg/ml)	40.72 ± 4.67	85.14 ± 2.92	157.80 ± 8.68	< 0.01
apelin-13/APJ ratio	17.32 ± 1.95	16.81 ± 0.63	16.56 ± 0.77	0.90
LAC(mmol/L)	0.73 ± 0.05	2.58 ± 0.14	3.48 ± 0.28	< 0.01
PCT(ng/mL)	0.23 ± 0.02	27.90 ± 2.71	64.71 ± 7.38	< 0.01
CRP(ng/L)	4.50 ± 0.36	110.17 ± 4.47	109.96 ± 7.15	< 0.01
IgG(mg/dL)	1138.25 ± 34.28	1016.26 ± 29.44	999.11 ± 57.49	0.16※
CD4 T cells(number/uL)	795 ± 38.87	324.75 ± 19.06	233.14 ± 22.21	< 0.01
SOFA score	/	6.74 ± 0.27	9.58 ± 0.59	< 0.01
CTNI(ng/ml)	/	0.88 ± 0.11	2.95 ± 0.32	< 0.01
BNP(pg/mL)	/	298.5 ± 31.43	386.84 ± 73.19	0.20
EF(%)	/	54.44 ± 0.88	40.72 ± 1.63	< 0.01
28-day mortality, n(%)	/	37(21.5)	35(53.0)	< 0.01
Mechanical ventilationn n (%)	/	61(35.5)	48(72.3)	< 0.01
Main diagnosis n (%)				
Pneumonia	/	112(65.12)	47(71.21)	0.37
IAI		26(15.12)	9(13.63)	0.77
USI		12(6.98)	5(7.58)	0.87
CNSI		13(7.56)	4(6.06)	0.91
SSTI		6(3.49)	0	–
DKA		3(1.74)	1(1.52)	0.66

The values are normally distributed data and expressed as mean and standard deviation,and number (%). WBC, white blood cells; APJ:The apelin receptor; LAC:Lactic acid; PCT: procalcitonin; CRP: C-reactive protein; SOFA score: Sequential Organ Failure Assessment Score; CTNI: Cardiac troponin I; BNP:type B natriuretic peptide; EF:ejection fraction; IAI: intra adominal infection; USI: urinary system infection; CNSI: central nervous system infection; SSTI: skin/soft tissue infection; DKA: diabetic ketoacidosis. ※LgG levels had difference in sepsis and conctrol ($P < 0.01$),but not sepsis and spetic shock($P = 0.99$).

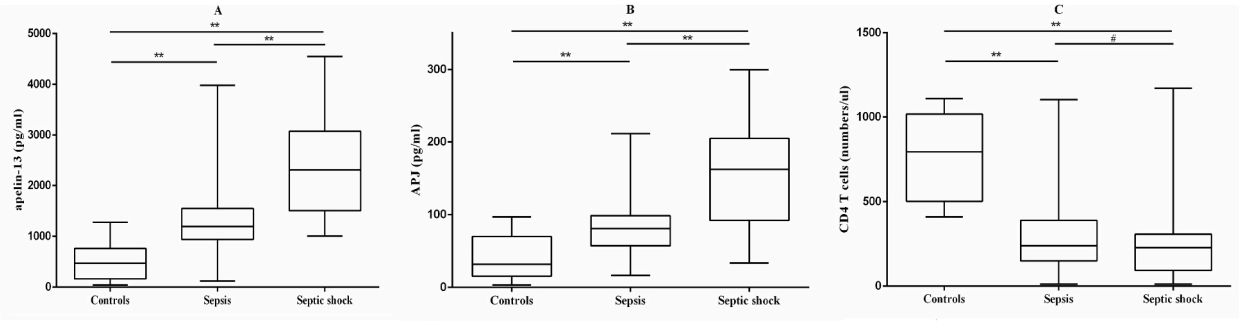

Fig. 1. Apelin-13, APJ and CD4$^+$ T cells levels in control and sepsis patients. Lines denote median values, boxes represent 25th to 75th percentiles and whiskers indicate the range. The signature elevation of apelin-13 (A), APJ (B) in sepsis patients compared with healthy control, but reduction of the CD4$^+$ T cells (C). Apelin-13 (A) and APJ (B) were significantly higher in septic shock than in sepsis, and CD4$^+$ T cells (C) were lower. (**P < 0.01, #P = 0.045).

13/APJ ratio, CRP, IgG, and BNP levels were not significantly differences in septic shock than in sepsis (all P > 0.05).

3.3. Apelin-13, APJ, and CD4$^+$ T cells levels in non-survivors and survivors

In the cohort under study, the survivors group comprised 166 patients, while the non-survivors group consisted of 72 patients. Apelin-13 levels (Fig. 2A) and APJ expression (Fig. 2B) exhibited significant elevation in non-survivors compared to survivors (both p < 0.01). Levels of CD4$^+$ T cells (Fig. 2C) were observed to be significantly reduced in non-survivors compared to survivors (p < 0.01). Additionally, non-survivors exhibited higher levels of PCT, lactate, and SOFA scores in comparison to survivors (p < 0.01). However, no significant differences were noted between the two groups regarding the apelin-13/APJ ratio, CRP, and IgG levels, as depicted in Table 2.

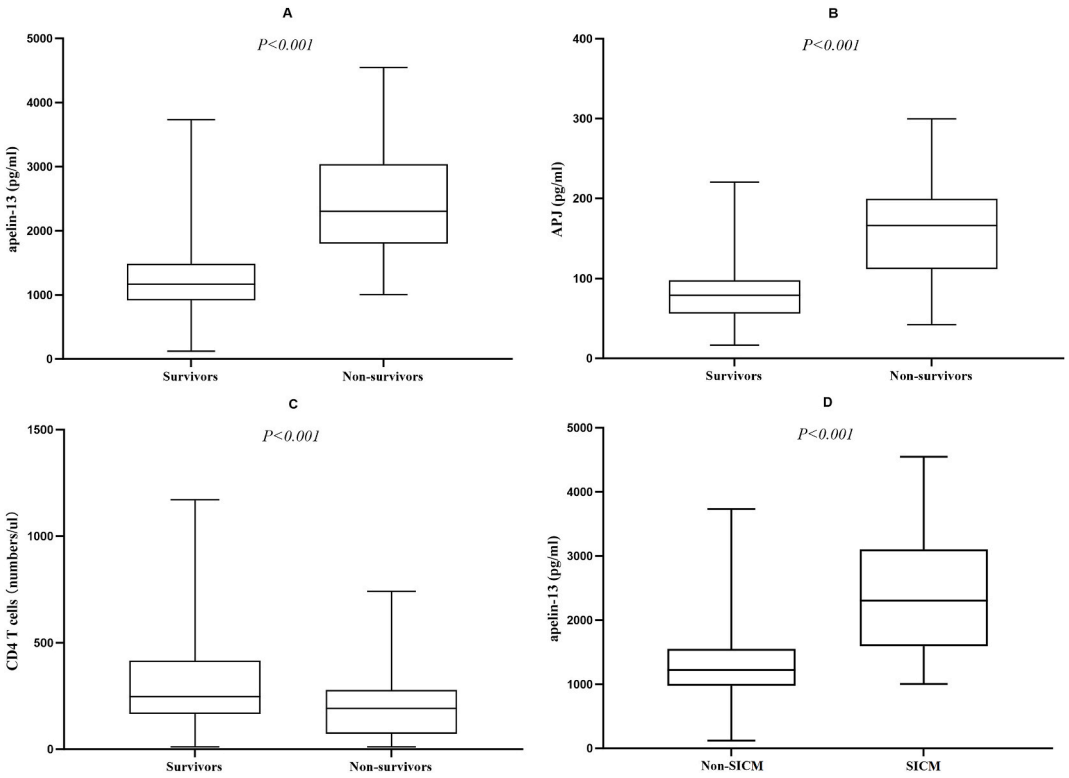

Fig. 2. Apelin-13, APJ and CD4$^+$ T cells levels in non-survivors and survivors and apelin-13 levels in Sepsis-Induced Cardiomyopathy (SICM) and Non-SICM Patients. Lines denote median values, boxes represent 25th to 75th percentiles and whiskers indicate the range. The signature elevation of apelin-13 (A), APJ (B) in non-survivors compared with survivors, but reduction of the CD4$^+$ T cells (P < 0.001) (C). Apelin-13 were significantly higher in SICM than in non-SICM patients (P < 0.001) (D).

3.4. Predictive value of Apelin-13 and APJ for 28-day mortality

In predicting 28-day mortality among septic patients, the area under the AUC for apelin-13 was calculated as 0.885, and for APJ, it was 0.871. These values surpassed those obtained for PCT (0.730; $p < 0.05$) and SOFA scores (0.726; $p < 0.05$). Fig. 3A illustrates the ROC curves of these biomarkers for predicting 28-day mortality. Using a threshold of 1768.38 pg/ml for apelin-13, our study aimed to predict 28-day mortality in patients with sepsis. The sensitivity achieved was 77.78%, with a specificity of 89.75%. Additionally, the PPV was 76.71%, and the NPV stood at 90.30%. For the APJ, employing a threshold of 99.59 pg/ml, our analysis revealed a sensitivity of 80.56% and a specificity of 83.13%. Furthermore, the PPV was calculated as 67.44%, while the NPV reached 90.79%. Tables 3 and 4 present the detailed results.

In our study, we observed a total of 72 patient fatalities within the 28-day follow-up period. Our objective was to investigate the prognostic value of apelin-13 and APJ in predicting 28-day survival among sepsis patients. We categorized sepsis patients into high and low groups based on the established cutoff values for apelin-13 (1768.38 pg/ml) and APJ (99.59 pg/ml). In accordance with Fig. 4, patients exhibiting elevated levels of apelin-13 and APJ demonstrated a significantly lower 28-day survival rate compared to those with lower levels of apelin-13 (Fig. 4A) and APJ (Fig. 4B).

3.5. Apelin-13 and APJ as independent predictors of 28-day mortality

Apelin-13, APJ, $CD4^+$ T cells, PCT, Lactate, IgG and the SOFA score were incorporated into a multivariate logistic regression analysis aimed at identifying independent predictors of 28-day mortality. Binary logistic regression analysis indicated that apelin-13 ($B = 0.002$, odds ratio (OR) $= 1.002$, $p = 0.004$), APJ ($B = 0.009$, $OR = 1.009$, $p = 0.016$), $CD4^+$ T cells ($B = -0.006$, $OR = 0.995$, $p = 0.004$), and lactate levels ($B = 0.327$, $OR = 1.386$, $p = 0.004$) emerged as independent predictors of 28-day mortality among septic patients. Conversely, PCT ($B = 0.004$, $OR = 1.004$, $p = 0.467$), IgG ($B = -0.001$, $OR = 0.999$, $p = 0.181$), and SOFA scores ($B = 0.086$, $OR = 1.090$, $p = 0.156$) did not exhibit significant associations (Table 5).

3.6. Apelin-13 level in sepsis-induced cardiomyopathy (SICM) patients and Non-SICM patients

In this study, 59 (24.7%) septic patients developed SICM. In patients with SICM, elevated levels of apelin-13 were observed compared to those in non-SICM patients ($p < 0.01$). As depicted in Fig. 3B, the AUC of apelin-13 for predicting SICM in septic patients was 0.871, which outperformed the AUC values of CTNI (0.741) and BNP (0.829) (Fig. 3B and Table 3).

4. Discussion

In recent decades, it is evident that sepsis is not only a symptom of complex infection but also a result of a compromised immune response to such infections [18,19]. In light of this, our study focused on the immune status of sepsis patients, recognizing the importance of early diagnosis and precise risk stratification for timely intervention. Consequently, there is a pressing need for biomarkers that can enhance early diagnosis and risk assessment. PCT serves as a commonly used clinical biomarker in patients with sepsis. In our study, we utilized PCT as a comparative variable alongside apelin-13 and APJ. In line with previous research [11], our investigation revealed heightened concentrations of apelin-13 in the peripheral blood of sepsis patients. Furthermore, a positive correlation was noted between apelin-13 levels and the severity of the disease.

Currently, sepsis is considered a dynamic syndrome characterized by concurrent proinflammatory and compensatory anti-inflammatory stages. This progression leads to an immunological condition recognized as compensatory anti-inflammatory response syndrome (CARS) [18,20]. Among peripheral lymphocyte subsets, $CD4^+$ T cells serve as key regulators in coordinating immune responses, exerting influence on both innate and adaptive immune cells via the secretion of cytokines and engagement in cell-to-cell interactions [21]. In our investigation, it was observed that $CD4^+$ T cell counts exhibited a notable reduction in the sepsis group in contrast to the control group ($p < 0.01$), with a progressive decline correlating with the severity of sepsis. In addition, among the sepsis patient cohort, the levels of $CD4^+$ T cells exhibited a significant decrease in the deceased group compared to the survivors (p

Table 2
Clinical data characteristics in survivor and non-survivor groups.

	survivor group n = 166	non-survivor group n = 72	P value
apelin-13(pg/ml)	1223.71 ± 40.28	2391.97 ± 98.31	<0.01
APJ(pg/ml)	80.42 ± 2.68	162.64 ± 7.53	<0.01
apelin-13/APJ ratio	16.87 ± 0.59	16.41 ± 0.92	0.37
LAC(mmol/L)	2.38 ± 0.10	3.86 ± 0.32	<0.01
PCT(ng/mL)	28.16 ± 2.85	60.8 ± 36.81	<0.01
CRP(ng/L)	106.9 ± 24.53	117.5 ± 6.84	0.54
IgG(mg/dL)	1019.33 ± 29.76	993.46 ± 29.76	0.83
CD4 T cells(number/uL)	321.81 ± 18.30	194.38 ± 16.15	<0.01
SOFA score	6.56 ± 0.28	9.61 ± 0.44	<0.01

The values are normally distributed data and expressed as mean and standard deviation. APJ: the apelin receptor; LAC:Lactic acid; PCT: procalcitonin; CRP: C-reactive protein; SOFA score: Sequential Organ Failure Assessment Score.

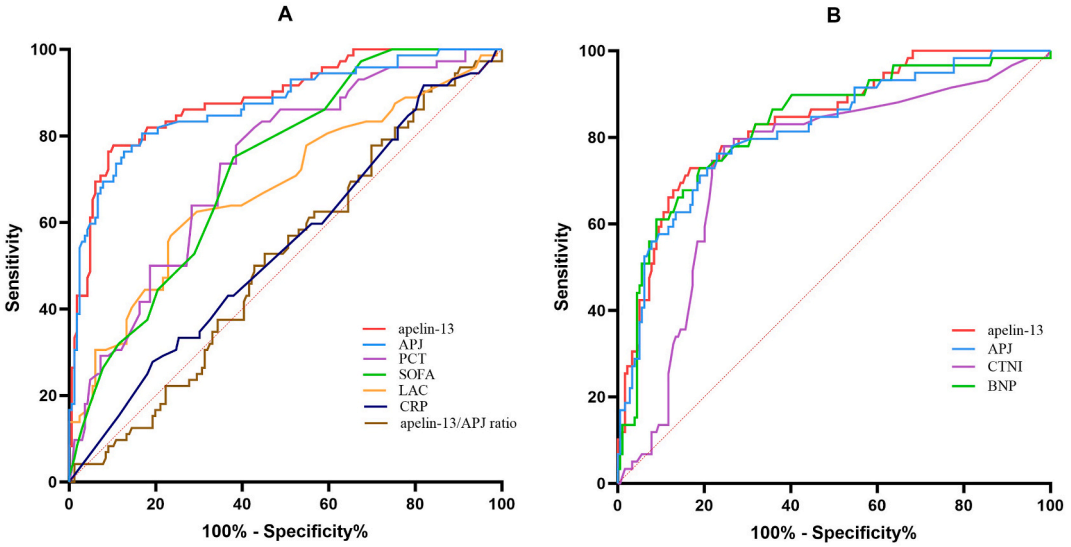

Fig. 3. Receiver operating characteristic curves of apelin-13, APJ, apelin-13/APJ, PCT, CRP, LAC, SOFA score for predicting 28-day mortality in septic patients (A). Receiver operating characteristic curves of apelin-13, APJ, CTNI and BNP for diagnosis SICM (B). APJ: the apelin receptor; LAC: Lactic acid; PCT: procalcitonin; CRP: C-reactive protein; SOFA score: Sequential Organ Failure Assessment Score; CTNI: Cardiac troponin I; BNP:type B natriuretic peptide.

Table 3
Areas under the curve of various parameters for predicting 28-day mortality in septic patients and for SICM.

	Variable	AUC	Standard error	P value	95% Confidence Interval	
					Lower limit	Upper limit
28-day mortality	Apelin-13	0.885	0.024	<0.01	0.838	0.933
	APJ	0.871	0.027	<0.01	0.818	0.924
	apelin-13/APJ	0.485	0.04	0.705	0.407	0.562
	PCT	0.730	0.034	<0.01	0.663	0.797
	CRP	0.542	0.041	0.307	0.462	0.622
	LAC	0.680	0.040	<0.01	0.602	0.757
	SOFA score	0.726	0.033	<0.01	0.661	0.791
SICM	Apelin-13	0.871	0.026	<0.01	0.821	0.922
	APJ	0.820	0.033	<0.01	0.755	0.884
	CTNI	0.741	0.038	<0.01	0.666	0.815
	BNP	0.829	0.032	<0.01	0.766	0.892

AUC, area under the receiver operating characteristic curve. APJ:the apelin receptor; LAC:Lactic acid; PCT: procalcitonin; CRP: C-reactive protein; SOFA score: Sequential Organ Failure Assessment Score; SICM:Sepsis-Induced Cardiomyopathy. CTNI: Cardiac troponin I; BNP:type B natriuretic peptide.

Table 4
Performance of multivariable models for predicting 28-day mortality in septic patients.

	Variable	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
28-day mortality	apelin-13	1768.38	77.78	89.75	76.71	90.30
	APJ	99.59	80.56	83.13	67.44	90.79
	PCT	20.36	77.78	61.45	46.67	86.44

NPV, negative predictive value; PPV, positive predictive value. APJ:the apelin receptor; PCT: procalcitonin.

< 0.01). The reduction of lymphocytes in sepsis is associated with lymphocyte apoptosis, decreased thymic export, and increased $CD4^+$ T cell senescence, resulting in a more pronounced decrease in $CD4^+$ T cells [22]. Furthermore, humoral immunity, comprising antibodies as pivotal components, plays a critical role in the organism's defense against pathogens [23]. Research has suggested a correlation between IgG levels and mortality among septic patients. Nevertheless, these studies have also indicated that diminished IgG levels fail to adequately distinguish between non-survival and survival in individuals with severe sepsis and septic shock [24]. Consistent with these findings, our study identified lower IgG levels among sepsis patients in comparison to controls ($p < 0.01$). However, we did not observe any notable distinctions in IgG levels between sepsis and septic shock cohorts or between deceased and

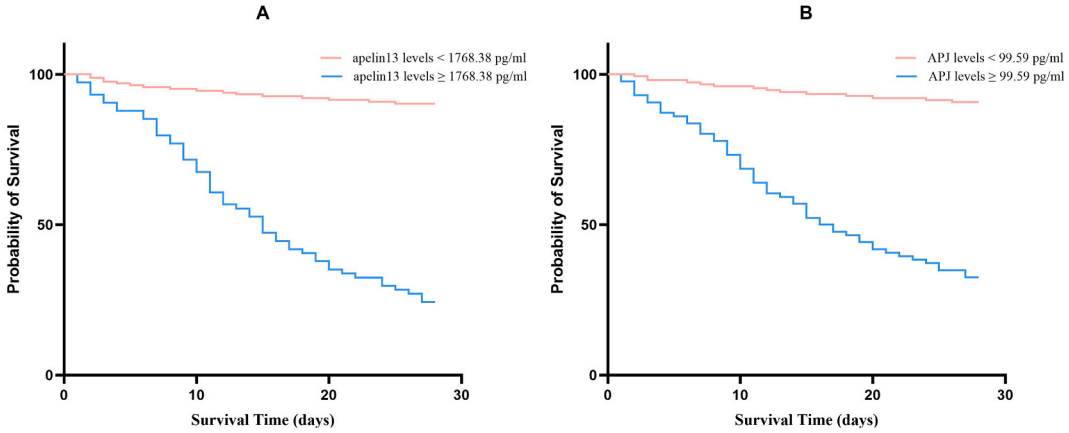

Fig. 4. Predictive significance of apelin-13 and APJ in 28-day survival in sepsis patients. Sepsis patients with high levels of apelin-13 (A) and APJ (B) were worse than those with low levels.

Table 5
Independent factors predicting 28-day mortality in septic patients.

	Variable	B	S.E.	Wald	P value.	Odds ratio	95% C.I.for EXP(B)	
							Lower limit	Upper limit
28-day mortality	Apelin-13	0.002	0.001	8.173	0.004	1.002	1.001	1.004
	APJ	0.009	0.012	0.531	0.016	1.009	0.985	1.033
	Apelin-13/APJ ratio	−0.062	0.076	0.679	0.410	0.939	0.810	1.090
	LAC	0.327	0.112	8.446	0.004	1.386	1.112	1.728
	PCT	0.004	0.005	0.529	0.467	1.004	0.994	1.014
	IgG	−0.001	0.001	1.788	0.181	0.999	0.998	1.000
	CD4 T cells	−0.006	0.002	8.493	0.004	0.995	0.991	0.998
	SOFA	0.086	0.061	2.008	0.156	1.090	0.968	1.228
	Constant	−4.621	1.655	7.799	0.005	0.010		

APJ:the apelin receptor; LAC:Lactic acid; PCT: procalcitonin; SOFA score: Sequential Organ Failure Assessment Score.

surviving subjects.

Apelin, a peptide hormone, has recently been recognized as the endogenous ligand of APJ (apelin receptor) [6]. Synthesized from a 77-amino acid precursor called preproapelin, apelin undergoes processing to yield several fragments, including apelin-12, apelin-13, apelin-17, and apelin-36, each exerting distinct activities in different tissues [8,25,26]. Apelin holds significance as an adipokine, with plasma apelin primarily sourced from the vascular endothelial cell lining found in several anatomical locations within the human body, such as the heart, kidney, large conduit vessels, adipocytes, and others [27]. APJ, a prototypical G protein-coupled receptor comprising 380 amino acids and characterized by seven transmembrane domains, exhibits widespread distribution throughout the body [28]. A strong link between apelin and inflammation exists, as demonstrated by Pan et al. [29], who revealed that apelin directly suppresses the synthesis of macrophage MCP-1 and IL-8 after stimulating peritoneal macrophages from rats with LPS. In septic rats, apelin reduced inflammatory factors such as MCP-1 and IL-8. It is postulated that the upregulation of apelin during inflammatory states represents a compensatory response, but this increase differs from the rise in inflammatory factors. Studies have demonstrated that apelin exhibits properties that include anti-inflammatory, anti-infectious, and inhibitory effects on the release of inflammatory mediators [8]. Our investigation revealed elevated levels of apelin-13 and APJ among participants diagnosed with sepsis compared to those in the control group ($p < 0.01$). And the levels were significantly higher in individuals with septic shock when compared to those with sepsis alone ($p < 0.01$). In the sepsis patient cohort, we observed a significant increase in apelin-13 and APJ levels among individuals who did not survive compared to those who did ($p < 0.01$). Notably, there were no notable distinctions in the apelin-13/APJ ratio between the healthy control and sepsis groups or between those who survived and those who did not. Because both Apelin and APJ increased in the same magnitude in groups. Both Apelin and APJ in sepsis (compared to normal) increased 2–2.5 fold, while both increased about 4 fold in septic shock (compared to normal). We hypothesize that due to the regulatory effects of Apelin-APJ binding on multiple biological systems [27], the apelin-13/APJ ratio did not differ significantly between groups. Despite $CD4^+$ T cell reductions suggesting immunosuppression in the septic shock group, why did apelin-13, an inflammation-inhibiting factor, increase? In sepsis, a cytokine storm leads to significant apoptosis of innate immune cells, resulting in a continuous, uncontrolled anti-inflammatory response termed CARS [30]. This stage of pathology is marked by extensive immune cell apoptosis, suppressing monocyte macrophages' ability to promote the release of inflammatory cytokines [30]. Immunosuppression renders the clearance of septic infection less efficient, exposing patients to secondary infections and reactivation of underlying infections, significantly increasing mortality rates among sepsis patients [31]. Given the pathogenesis of sepsis, the elevated levels of circulating apelin-13 and APJ in our study aligned with the clinical

severity of sepsis. Consequently, the elevation of apelin-13 and APJ has become recognized as a valuable indicator for evaluating the severity of sepsis.

The ED frequently acts as the primary site for assessing and stratifying the risk of septic patients, which is pivotal for prompt intervention. Our study concentrated on the initial prognostic evaluation of individuals with sepsis. The SOFA score was developed for the evaluation of organ dysfunction associated with sepsis, a factor intricately linked to the prognosis of septic patients. A previous study on septic patients demonstrated the prognostic stratification ability of the SOFA score [32]. Our findings indicate that apelin-13 or APJ exhibit superior prognostic value compared to PCT and the SOFA score. Notably, apelin-13 or APJ, rather than PCT or the SOFA score, were identified as strong independent predictors of mortality within 28 days in patients with sepsis. The significant differences in apelin-13, APJ, and CD4 Th cell levels between survivors and non-survivors underscore their prognostic potential. Our extensive ROC analysis on a significant cohort of septic patients admitted to the ED revealed that apelin-13 or APJ exhibited superior sensitivity and specificity for early 28-day mortality diagnosis compared to PCT. In our investigation, 72 patients succumbed during the 28-day follow-up period, with the majority of fatalities observed among individuals exhibiting elevated levels of apelin-13 and APJ. These findings collectively emphasize the potential of apelin-13 or APJ as markers for identifying septic patients who may face increased short-term mortality upon admission to the ED.

In septic shock, multiple organ failure emerges as a prominent contributor to mortality, emphasizing the imperative of safeguarding the cardiovascular system [33]. SICM frequently co-occurs with sepsis, compounding mortality risk. In this context, infusion of apelin-13 offers unique and optimized hemodynamic support, alongside cardioprotective effects and the modulation of circulatory inflammation, ultimately extending survival [34]. Apelin primarily exerts its effects on the cardiovascular system, particularly when myocardial injury occurs, by promoting apelin secretion to inhibit cardiac cell apoptosis, enhance myocardial contraction, and reduce myocardial injury [12]. Previous study has shown that the exogenous administration of apelin elicits positive inotropic effects in both healthy myocardium and in hearts affected by heart failure [29]. The apelinergic system emerges as a viable option for addressing low-output septic shock and alleviating hemodynamic disturbances, offering an alternative to the conventional use of catecholamines [34].

In previous research, exogenous administration of apelin has shown positive inotropic effects on both normal and failing hearts [29], suggesting its potential as an alternative to catecholamines for managing low-output septic shock and alleviating hemodynamic disturbances [34]. In our investigation, we observed that 59 out of 239 septic patients (24.7%) met the criteria for SICM according to established guidelines [17]. Notably, the levels of Apelin-13 were found to be significantly elevated in patients with SICM compared to those without ($p < 0.01$). Our analysis of the AUC for apelin-13 in predicting SICM revealed superior prognostic capability compared to other biomarkers. Our study thus established apelin-13 as a biomarker for sepsis-induced cardiomyopathy. Given the unique combination of inotropic and anti-inflammatory effects of apelin, we speculate that apelin may serve as an endogenous cardioprotective agent, counteracting myocardial impairment in sepsis. As a result, apelin has surfaced as a promising candidate for therapeutic intervention in cases of sepsis and septic shock [29].

5. Limitations

Several limitations should be considered in this study. The study was carried out at two hospitals and did not include an evaluation against alternative severity score systems or biomarkers. Due to the restricted sample size, certain findings might not entirely reflect the actual scenario. The lack of continuous dynamic monitoring of inflammatory response cytokines in sepsis prevented the elucidation of the interplay between inflammatory response and immune function in sepsis. Treatment decisions and patient preferences may introduce confounding and bias.

6. Conclusions

Sepsis is associated with immune suppression. Apelin-13 and APJ have emerged as prospective biomarkers for the early diagnosis, risk stratification, and prognosis assessment among patients with sepsis. Additionally, Apelin-13 exhibits promise as a biomarker specifically for sepsis-induced cardiomyopathy.

Funding statement

This work was supported by Open Foundation from Beijing Key Laboratory of Cardiopulmonary Cerebral Resuscitation (2018XFNKFKT-02).

Declaration of ethics

The study was approved by the Hospital Institutional Review Board and the Ethics Committee of Beijing Chaoyang Hospital (no.2021-ke-686), and written informed consent was obtained from all participants or their relatives.

Additional information

None.

Data availability statement

If data is needed, application can be made to the corresponding author.

CRediT authorship contribution statement

Miaomiao Wang: Writing – original draft, Visualization, Software, Resources, Methodology, Formal analysis, Data curation. **Qian Gao:** Software, Methodology, Data curation. **Shubin Guo:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank the ED staff, Physical examination center of Beijing Chao-yang Hospital and Biochemistry Laboratory staff for their helpful contributions to this study.

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