Serum cholinesterase as a prognostic factor of mortality in children with sepsis

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Abstract

Introduction: Mortality in septic children continues to be high in Indonesia. Biomarkers for evaluating outcome of children with sepsis are important in deciding further diagnostic examinations or therapies. However, the serum cholinesterase predicting value for mortality in children is still unknown.

Objectives: To evaluate the ability of serum cholinesterase as a prognostic factor for mortality in children with sepsis.

Method: This was a prospective cohort study in the Paediatric Intensive Care Unit (PICU) at Sanglah Hospital, Bali, Indonesia. A total of 78 children aged 28 days to 18 years with sepsis were included and divided into survivor and non-survivor groups. Children with incomplete medical record data, severe protein energy malnutrition, inhibitor cholinesterase therapy, liver dysfunction, HIV infection, malignancy, diabetes mellitus and chronic kidney disease were excluded. Serum cholinesterase level was measured when sepsis was diagnosed. Statistical analysis used Chi-squared test or Mann-Whitney test for bivariate analysis and Poisson regression for multivariate analysis.

Results: Proportion of septic children aged below 5 years was 68.4% in non-survivor group and both groups were predominantly male. Serum cholinesterase ≤5413.5 U/L was associated with mortality in both bivariate analysis (relative risk 5.33 (2.518-11.297) and multivariate analysis (adjusted relative risk 5.294 (95% CI 2.213-12.662)). However, age ≤ 5 years, paediatric logistic organ dysfunction (PELOD) -2 score, and malnutrition were not associated with mortality.

Conclusions: Serum cholinesterase level was an independent prognostic factor for mortality in children with sepsis.

(Key words: Serum cholinesterase, Sepsis, Children, Mortality)

Introduction

Although sepsis is the main cause of morbidity and mortality in children, the diagnosis, treatment and predicting prognosis of sepsis is still challenging to most clinicians. A study in the United States of America showed that the incidence of sepsis has increased about 20 times in the last two decades and that it is responsible for 200,000 deaths per year[1]. Epidemiology data in Indonesia reported the prevalence of sepsis morbidity and mortality was 19.3% and 54% respectively, and that the most common source of infection was the respiratory system (40%)[2].

Studies have described several prognostic factors that may be associated with morbidity and mortality in septic children in the Paediatric Intensive Care Unit (PICU), ranging from clinical to laboratory. Significance of clinical prognostic factors such as age, malnutrition, organ dysfunction and severity of sepsis have been previously reported[1,3], as well as early markers such as procalcitonin, C-reactive protein, Interleukin-6, and Interleukin-8. Although many studies have identified prognostic factors for poor outcomes in septic patients in the PICU, evidence on these factors in Indonesia, particularly Bali, is still limited. Biomarkers for assessment of outcome of septic patients could facilitate clinical decision making in determining further diagnostic or therapeutic measures, optimising available resources and providing appropriate counseling for patients or their families[4].

Cholinesterase is synthesized in the liver and catalyses the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid, which is necessary for the cholinergic nerves to return to their resting position after activation[4]. Cholinergic agonists play a role in stimulating the release of

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acetylcholine and its function as an anti-inflammatory agent when inflammation occurs. Stimulation of the vagus prevents the harmful effects of cytokine release in experimental sepsis, endotoxaemia, ischaemia / reperfusion injury, haemorrhagic shock, arthritis, and other inflammatory syndromes[3]. The cholinergic anti-inflammatory pathway controls the production of cytokines during an inflammatory response in the body. Prior evidence showed that serum cholinesterase has an important role in the inflammatory response and also reported it to be a prognostic marker for septic patients[5]. Decreased cholinesterase activity is associated with severity and mortality in critically ill patients, with varying prognostic values for different diseases[6].

Objectives

To evaluate the significance of serum cholinesterase as a prognostic factor for mortality in children with sepsis.

Method

A single-centre prospective cohort study was conducted in the PICU of Sanglah Hospital, Bali, Indonesia from August 2019 to May 2021.

Inclusion criteria: Children aged 28 days to 18 years when admitted to the PICU with an established diagnosis of sepsis in the PICU.

Exclusion criteria: Children with severe protein energy malnutrition, inhibitor cholinesterase therapy, liver dysfunction, HIV infection, malignancy, diabetes mellitus, chronic kidney disease and incomplete medical record data.

Sample size: Consecutive sampling was done. Based on the sample size formula of estimated proportion of binary outcomes and unpaired categorical samples, we calculated the sample needed from each variable. We used the proportion of mortality patients with low cholinesterase level as 46% and the proportion of mortality patients without risk as 19%. Based on the literature, the proportion of patients with depression without each risk factor was age (36%), maternal education (28%), orphan status (40%), CD4 count (42%), caregiver (29%), HIV clinical stage (40%), hospitalization (80%) as the P2 value. Based on the sample sizes obtained above, the number of samples was used, namely a total of 78 samples.

Serum cholinesterase was measured at the time of admission. Patients were divided into two levels of cholinesterase, low (≤5413.5 U/L) and high (>5413.5 U/L)) and were followed for 30 days or until each patient was discharged or died. Patients were assigned into survivor and non-survivor groups. Data for each patient were collected from medical records subsequently; age and gender, nutritional status, organ dysfunction, sepsis severity, blood culture, Paediatric Logistic Organ Dysfunction (PELOD)-2 score, length of stay, history of antibiotic use, ventilator use, and procalcitonin level, were recorded in the case report form and questionnaire.

Sepsis was diagnosed by the presence of infection, including predisposing factors, signs or evidence of infection (procalcitonin ≥2 ng/ml), inflammation response and PELOD-2 score ≥ 7 without lactate result[2]. Severity of sepsis was divided into two categories, sepsis, and septic shock. Septic shock was defined as sepsis requiring vasopressor therapy to maintain mean arterial pressure (MAP) ≥65 mmHg or lactate level >2 mmol/L (18mg/dL) despite adequate fluid resuscitation[7]. Serum cholinesterase level was obtained from septic subjects at the time of admission. Organ dysfunction was measured by PELOD-2 score, which consists of ten variables for five vital organ systems, namely neurologic, cardiovascular, renal, respiratory, and haematologic. PELOD-2 is a continuous scale and is measured in the first 24 hours of sepsis onset. Nutritional status was measured based on actual subject weight, compared to ideal body weight (based on WHO curve) by Waterlow.

Ethical issues: The study was approved by Research Ethics Committee at the Faculty of Medicine Udayana University/Sanglah Hospital, Denpasar (2307/UN.14.2.2.VII.14/LP/2019) and signed informed consent was obtained from all parents/caregivers.

Statistical analysis: Collected data were analysed using SPSS for Windows. Bivariate analysis was used to evaluate the association of outcome (mortality) with each risk factor using Chi-squared test. Optimum cutoff cholinesterase level was estimated by Receiver Operating Characteristic (ROC) curve and Youden Index. Poisson regression was used for multivariate analysis. p-value less than 0.05 was considered significant.

Results

From August 2019 to May 2021, 112 children were diagnosed with sepsis and 78 met the inclusion criteria. The process of subject recruitment is illustrated in Figure 1.

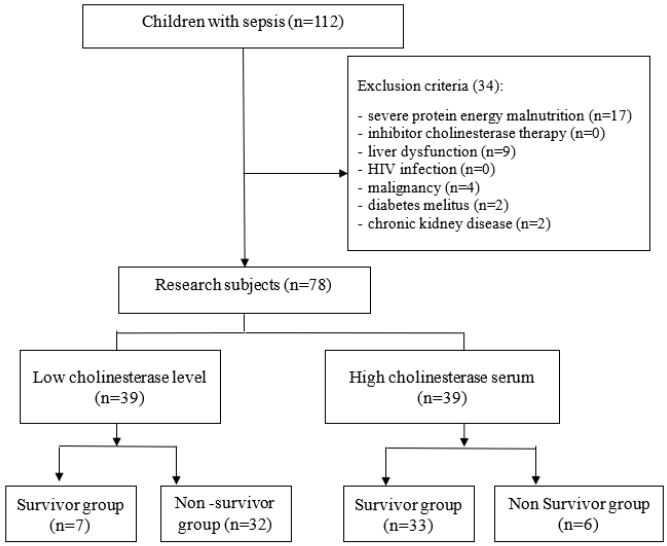

Figure 1: ***Study flow chart***

In the non-survivor group, 26 (68.4%) septic children were less than 5 years old. Patients in both non-survivor and survivor groups were predominantly male (57.9% and 57.5% respectively). Characteristics of the research sample according to the serum cholinesterase level are shown in Table 1.

Table 1: ***Characteristic of subjects in the research sample according to serum cholinesterase level (n=78)***

Characteristic	Low cholinesterase level *(n=39)*	High cholinesterase level *(n=39)*
Age - n (%)		
< 5 years	26 (66.7)	27 (69.2)
≥5 years	13 (33.3)	12 (30.8)
Gender- n (%)		
Male	22 (56.4)	23 (59.0)
Female	17 (43.6)	16 (41.0)
Nutritional status - n (%)		
No malnutrition	25 (64.1)	28 (71.8)
Malnutrition	14 (35.9)	11 (28.2)
Sepsis severity - n (%)		
Sepsis	19 (48.7)	20 (51.3)
Septic shock	20 (51.3)	19 (48.7)
Blood culture - n (%)		
Positive	11 (28.2)	7 (17.9)
Negative	28 (71.8)	32 (82.1)
PELOD-2 score - n (%)		
<7	17 (43.6)	18 (46.2)
≥7	22 (56.4)	21 (53.8)
Laboratories - median (IQR)		
Procalcitonin level (ng/ml)	13.22 (70.3)	7.4 (34.96)
Length of stay (days) - median (IQR)	15 (23)	10 (17)
History of antibiotics - n (%)		
Yes	15 (38.4)	17 (43.6)
No	24 (61.6)	22 (56.4)
Ventilator use - n (%)		
Yes	25 (64.1)	27 (69.2)
No	14 (35.9)	12 (30.8)
Outcome – n (%)		
Survivor	7 (17.9)	33 (84.6)
Non-survivor	32 (82.1)	6 (15.4)

Bivariate analysis indicated that three prognostic factors were not significantly associated with mortality. These were age <5 years, nutritional status and PELOD-2 score, as shown in Table 2.

Table 2: ***Bivariate analysis of prognostic factors associated with mortality***

Prognostic factor	Non-survivor n (%)	Survivor n (%)	RR (95% CI)	p-value
Age <5 years	26 (68.4)	27 (67.5)	1.022 (0.625-1.671)	0.931
Malnutrition	13 (34.2)	12 (30.0)	0.907 (0.566-1.455)	0.690
PELOD-2 score ≥7	24 (63.2)	19 (47.5)	0.717 (0.411-1.164)	0.165
Cholinesterase level ≤ 5413.5	32 (84.2)	07 (17.5)	5,33 (2.518-11.297)	0.001*

**Significant p <0.05 by Chi-square test*

Prognostic factors that had p-values less than 0.25 in bivariate analysis (PELOD-2 score, and cholinesterase level ≤5413.5 U/L) were included in multivariate analysis. Among the prognostic factors included, only one was independently significantly associated with sepsis mortality as shown in Table 3.

Table 3: ***Multivariate analysis of risk factors of associated with mortality***

Variable	aRR (95% CI)	p-value
PELOD-2 score	0.743 (0.384-1.436)	0.376
Cholinesterase ≤ 5413,5	5.294 (2.213-12.662)	0.001*

**Significant p <0.05 by Poisson Regression*

Discussion

Sepsis is diagnosed in more than 4% of hospitalized patients less than 18 years old and 8% of patients admitted to PICUs in high-income countries[8]. In our study, the mortality rate in septic children was 48.7%. Majority of children who died from sepsis had refractory shock and/or Multiple Organ Dysfunction Syndrome (MODS). This is similar to a study by Weiss SL, *et al*[9] where the mortality of septic children ranged from 4% to 50%, depending on the severity of illness, risk factors, and geographic location.

In our study, the gender of septic children was predominantly male (57.7%) with a male: female ratio of 1.36:1. This is similar to previous studies which found that the incidence of sepsis was higher in males (52% and 61%) with male: female ratios of 1.08:1 and 1.24:1[10,11]. Male dominance in critically ill patients may be caused by gender susceptibility influenced by hormones in the response to infection, presence of comorbidities, or the source of infection. This is related to the concept that X-linked immunoregulatory genes are the main factors that confer immunological superiority in females. This gene can provide resistance to many disorders that the immune response can prevent[12]. In our study, organ dysfunction was found in those who died. The most common organ dysfunction was respiratory (81.5%), followed by central nervous system (65.7%), haematological (26.3%), renal (18.4%) and cardiovascular (18.4%). This is similar to a study by Watson RS, *et al*[13] where organ dysfunctions were frequently found in the respiratory, cardiovascular, central nervous system, renal, and haematological systems[13].

The prognostic factors associated with sepsis mortality are hard to identify, especially in the early stage of sepsis. Several biomarkers such as procalcitonin, C-reactive protein, interleukin-6 and interleukin-8 are used to determine the prognostic mortality of sepsis in children. These biomarkers could also facilitate clinical decision making in terms of determining further diagnostic or therapeutic measures, optimising available resources and providing appropriate counselling to patients or their families. However, the results of these biomarkers were not satisfactory.

Serum cholinesterase is one of the biomarkers that has been studied in predicting mortality of sepsis in children. In our study, we found the optimum cut-off point of serum cholinesterase level was 5413.5 U/L and low serum cholinesterase was significantly associated with mortality with RR 5.294 (95% CI 2.213-12.662; p=0.001). A study by Zivkovic AR, *et al*[14] found that the level of serum cholinesterase is a biomarker that can predict the outcome of sepsis patients, and the recommended cut-off value was less than 1661 U/L. A similar study by Bahloul M, *et al*[5] found that serum cholinesterase level less than 4000 U/L could predict poor outcome in septic patients. A study by Peng ZL, *et al*[6] reported that serum cholinesterase was an independent prognostic factor in mortality in adult septic patients with an OR value of 2.11 (95% CI 1.37-3.21; p=0.0008).

The PELOD score is a frequently used scoring system that describes multiple organ dysfunction in paediatric patients. This score was first developed in 1999 and has since been modified to PELOD-2, which assesses the severity of MODS with good validity[15]. Different cutoff points have been reported in the literature and this may be due to differences in patient characteristics, facilities, and staff capability at each centre. A PELOD-2 cutoff point of ≥ 11 was set as the criterion for predicting life-threatening organ dysfunction, though this contrasts with a multicentre study in Europe that reported a PELOD-

2 score ≥ 8 as the cutoff point[16]. Our study found a cutoff ≥7 without lactate to be not significantly associated with mortality in septic patients. Our study found that a PELOD-2 score ≥ 7 increased the risk of mortality, with RR 0.743 (95% CI 0.384–1.436). A higher PELOD-2 score indicates multiple organ dysfunction and poorer prognosis, and the most common organ dysfunction seen in septic patients was related to the respiratory system[16]. A study by Wati DK, *et al*[15] in the PICU at Sanglah Hospital found that 42.9% of patients admitted to the PICU had three organ dysfunctions, with a median PELOD-2 score of 6 (IQR 8), whilst 58.3% of those who had three organ dysfunctions died. This is similar to a study by Watson S, *et al*[18] which stated that the risk of death increased if there is organ failure, from 7% in one organ failure to 53.1% in 4 organ failures. They also stated that if there is multi-organ failure, the mortality rate can reach 80%[18]. These contradictory results may be because PELOD-2 score was only calculated once, within the first 24 hours of admission, which might not represent an optimal measurement of PELOD-2 score which changes over time.

Mechanical ventilation is used in 40-65% of all patients admitted to the intensive care unit[12]. In our study, 27 (71.1%) subjects died using mechanical ventilation in their care. The need for mechanical ventilation is the most common priority for intensive care[12]. Length of stay is an important predictor influencing the outcome of critically ill patients admitted to the PICU. Prolonged length of stay increases mortality. In our study, median length of stay in children with a death outcome was 14 days. Length of stay is influenced by age, comorbidities, hypermetabolism, organ failure, and nutritional deficiencies[19]. Blood cultures are particularly important for diagnosing sepsis and for establishing treatment or follow-up in patients with suspected sepsis. Ideally blood culture will give results after 48 hours from the examination[20]. Our study had positive blood cultures in 18.4% patients with a mortality outcome and 27.5% patients with a survival outcome. This low rate of positive blood cultures could be attributed to aggressive empiric outpatient infection treatment and early administration of antibiotic therapy before cultures were collected in patients with suspected sepsis. Other explanations include viral and fungal infections that cannot be cultured or incomplete and unsuitable available media[21].

Patient age has been considered as a prognostic factor for mortality among patients with sepsis in previous studies. However, our study found that age was not associated with the risk of mortality with RR 1.022 (95% CI 0.625-1.671). This contradictory result is supported by Villegas D, *et al*[22] who found that age less than 1-year increased mortality in children with sepsis[22]. These contradictory results may be due to the large variations in age groups and cutoffs used in prior studies and the wide age range in our study of 28 days to 18 years.

Malnutrition is associated with altered metabolism. In critically ill children, the body's response to disease can include a rapid decrease in metabolic rate, decrease in oxygen consumption, reduction in energy production, disturbance in hormonal regulation marked by a hyper-catabolism state, negative nitrogen balance, loss of weight and muscle mass, and alteration in carbohydrate metabolism. Malnutrition worsens the prognosis of severely ill children and contributes to higher mortality, longer length of hospital-stay and higher health cost[23]. In this study, malnutrition was not associated significantly with mortality in children with sepsis (RR 0.907; 95% CI 0.566–1.455). Villegas D, *et al*[22] found severe protein energy malnutrition increased the risk of mortality in children with sepsis. Risk of mortality in malnutrition increased 9.43 times than if there was no malnutrition. This result could be because severe protein energy malnutrition was not included in this study.

Our study had some limitations. The time of blood sampling was not immediately after the occurrence of sepsis; it was sampled in a wide time range up to 4 days after the onset of sepsis, so this could affect the results of the serum cholinesterase.

Conclusions

Serum cholinesterase level was an independent prognostic factor for mortality in children with sepsis.

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