Receptor-interacting protein kinase 3 has a good accuracy in predicting mortality of critically septic patients

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Abstract

***Objective*: Sepsis-related uncontrolled systemic inflammation may trigger necroptosis, which is correlated with receptor-interacting protein kinase 3 (RIPK3) level. A high level of RIPK3 is associated with organ dysfunction and thus, mortality. This study aimed to analyze the ability of RIPK3 level to predict mortality in septic patients.**

***Design*: This was a prospective cohort study.**

***Setting*: This study was conducted in intensive care unit from February until August 2019.**

***Patients and participants*: This study included patients aged 18 years or more who met the Sepsis-3 definition. Baseline demographic data were measured.**

***Interventions*: Blood was collected to measure RIPK3 at sepsis recognition. RIPK3 level was measured using enzyme-linked immunosorbent assay using Bio-Rad (Bio-Rad Laboratories, California, US). Hour-1 bundle resuscitation was performed on all participants. Participants were observed for 28 days for mortality. Data were analyzed using STATA program software.**

***Measurement and results*: A total of 59 subjects were analyzed. The cutoff point of RIPK3 level was 0.51 ng/ml with 92.5% sensitivity and 89.5% specificity in predicting mortality. RIPK3 level has an excellent performance with area under the receiver operating characteristic (ROC) curve (AUC) value of 0.925.**

***Conclusions*: RIPK3 level can be considered a useful tool to recognize high risk mortality among critically septic patients.**

Key words: Mortality, predictor, receptor-interacting protein kinase 3, sepsis.

Introduction

Sepsis is a life-threatening organ dysfunctions condition caused by a dysregulated host response to infection. (1) Sepsis still a major burden, especially in the intensive care unit (ICU). One-third of patients who died in the hospital were diagnosed with sepsis. (2) In 2014, septic cases were found in 173,690 cases among 2.9 million adult patients admitted to study hospitals and contributed to a high mortality rate. (3) In developing countries, such as Indonesia, sepsis-related mortality is still high despite maximal effort.

Inflammatory marker such as C-reactive protein (CRP) could predict the septic patients' mortality accurately. (4) Plasma cytokine such as tumor necrosis factor (TNF)-α was increasing significantly in patients with sepsis and septic shock. Anti TNF-α might decrease mortality risk in septic patients. Uncontrolled inflammation in sepsis may induce a proinflammatory mediator and trigger necroptosis, programmed cell death, and cause organ dysfunction or damage. (5) The term necroptosis was initiated after receptor-interacting serine/threonine protein kinase 1 (RIPK1) was recognized as an important regulator of inflammation, cell survival, and disease in the early 2000s. (6) Many studies supported receptor-interacting protein kinase 3 (RIPK3) as an end product of the necroptosis pathway. (6) RIPK3 was produced from TNF and inhi-

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bition of the caspase 8 enzyme. RIPK3 has clinical implications in septic patients. An animal study showed that RIPK3 deficiency was able to protect the subjects against sepsis and had potential to be a sepsis therapy target. (7) RIPK3 level can be used as a marker for predicting sepsis outcomes and identifying patients who will benefit from aggressive therapies. (8)

Sepsis-related mortality remains an unpredictable event. Our current knowledge of sepsis is still limited as there is no study to analyze the ability of RIPK3 level to predict mortality in septic patients. This study aimed to analyze the ability of those parameters to predict mortality in septic patients.

Materials and methods

This was a prospective cohort study. Participants who met eligible criteria were recruited and followed for 28 days. This study was conducted at ICU and resuscitation room from February to August 2019.

Seventy-two participants included in the study, thirteen were dropped due to inability to complete standardized protocol. Fifty-nine patients were analyzed. This study was conducted after obtaining clearance from Health Research Review Committee and recorded in ClinicalTrials.gov (NCT04169412). Participants in this study were recruited through non-probability consecutive samplings. Participants were at least 18 years old who met Sepsis-3 definition (suspected infection and Sequential [sepsis-related] Organ Function Assessment [SOFA] score were two points or more). Patients whose relatives declined for them to participate, were not treated in the ICU, were diagnosed more than 24 hours for sepsis, were pregnant or had brain death were excluded. Patients who did not complete standardized hour-1 protocol and could not be followed up with observation within 28 days were dropped from the study.

The primary outcome of this study was to determine the mortality rate in patients with sepsis. RIPK3 level were considered to be increased if beyond the cutoff level (0.51 ng/ml). Failed resuscitation was defined as an inability to achieve lactate level less than 2 mmol/l or lactate reduction more than 20% within 4-hour resuscitation. Dependent variable was mortality within 28 days. The confounding variables were comorbidities, age, and acute physiology and chronic health evaluation (APACHE II) score.

Blood collection was performed in participants who met eligible criteria. RIPK3 level was measured using enzyme-linked immunosorbent assay using Bio-Rad (Bio-Rad Laboratories, California, US).

The data was analyzed with the appropriate statistical test using the Stata/IC 15.0 for Mac (StataCorp, Texas, US). The characteristic variable was expressed according to their types (numerical and categorical) and data normality test. Data normality distribution was analyzed by using Kolmogorov Smirnov test. Variable in numerical were presented as mean±standard deviation for normal distribution or median (minimum-maximum) for not normal distribution and categorical variables were expressed in percentage. The results of data analysis will be presented in a table, graph, and text. Receiver operating characteristics (ROC) were used to determine the cutoff point of RIPK3 level to risk sepsis-related mortality. Cutoff point was the value that has optimal sensitivity and specificity.

Statistical analysis was divided into two stages. The first stage was descriptive analysis to assess the size of distribution (incidence) of the variable characteristics of the study participants. The second stage was inferential analysis to calculate the relationship between RIPK3 level to predict sepsis-related mortality. This study using Chi-square test for bivariate analysis while multivariate analysis using Cox regression time constant.

Authors tried to minimize the effect of bias by creating proper inclusion and exclusion criteria. Variables which have potential to influence the association between independent and dependent variables were analyzed. Those variables included age, comorbidities, and APACHE II score.

Results

From fifty-nine (59) participants, forty (40) participants died within the 28-day observation, yielding a mortality rate of 67.8% (**Figure 1**). Participants with a higher proportion of sepsis-related mortality were male, non-surgical diagnoses, had comorbidities, hypoalbuminemia (with albumin levels was less than 3.5 g/dl), higher SOFA and APACHE II scores, had secondary infection, were a patient from emergency room, had higher RIPK3, and lactate level (**Table 1**). Descriptively, this study found RIPK3 level in non-survival group were higher than survival group (1.27±0.31 vs 0.43±0.35 ng/ml).

The cutoff point of RIPK3 level was 0.51 ng/ml with 92.5% sensitivity and 89.5% specificity. The area under the receiver operating characteristic (ROC) curve (AUC) value of 0.925 indicated that RIPK3 had special accuracy as a predictor of sepsis-related mortality (**Table 2**).

Bivariate analysis resulted in significant relationship between group who had a RIPK3 level higher than 0.51 ng/ml (relative risks [RR]=5.86, $p<0.0001$, 95% confidence interval [CI]=2.066-16.610) and sepsis-related mortality.

Discussion

Necroptosis is a mode of regulated cell death with combination of apoptotic and necrotic. Morphologically it is presented as cell swelling followed by rupture of the plasma membrane. (9) In sepsis, necroptosis can be induced by systemic inflammatory response syndrome (SIRS). (5) SIRS produces the proinflammatory mediator TNF, which stimulates the necroptosis process, as well as other inflammatory processes and apoptosis. TNF receptor 1 (TNFR1) ligation by TNF recruits an initial complex consisting of the TNFR1-associated death domain (TRADD) protein and RIPK1. If caspase 8 activity were absent, deubiquitylated RIPK1 will recruit RIPK3 through auto-phosphorylated homotypic receptor interacting protein homology interaction motif (RHIM) and form a necrosome. In this complex, mixed-lineage kinase domain-like proteins (MLKL) are recruited and phosphorylated by RIPK3 and form active oligomers, which induce plasma membrane instability. At the end, cells will experience necroptosis and intracellular content leakage, including damage associated molecular patterns (DAMPs) inducing pro-inflammatory response. (6,9)

RIPK3 protein (a regulator of inflammation, cell survival, and disease) is needed for the occurrence of necroptosis. (9) RIPK3 level increases proportionally to the degree of sepsis inflammation. RIPK3 level in patients with severe sepsis and septic shock reach peak level at 72 hours and then decrease slowly. (8) Plasma RIPK3 levels 24 hours after sepsis were 7.9 (3.2-16.7) pg/ml, and 11.3 (1.2-19.2) pg/ml for severe septic patients, and 18.9 (3.6-32.5) pg/ml in septic shock patients. (8) RIPK3 levels in patients with severe sepsis and septic shock also increased significantly at various sampling times (p-value <0.05). (8)

This study found that RIPK3 levels were higher in non-survivor than survivor group (1.27±0.31 vs 0.43±0.35 ng/ml). This study found greater RIPK3 levels than previous studies. We assumed this was a result of the fact that this study only included septic patients treated in the ICU with high levels of organ dysfunction.

The study found a significant association between RIPK3 level and 28-day sepsis-related mortality (p<0.0001, 95% CI=2.066-16.610). Patients with sepsis and a RIPK3 level equal to or higher than 0.51 ng/ml had a 5.86 times higher risk of death within 28 days of recognition. The proportion of non-surviving subjects with a RIPK3 level ≥0.51 ng/ml (92.50%) was greater than patients with RIPK3 less than 0.51 ng/ml. Previous studies showed that there was a relationship between organ dysfunction or failure with mortality rate. Wang et al. showed there was a positive correlation between RIPK3 and organ dysfunction degree expressed as SOFA score (r=0.79, p=0.001). (8) RIPK3 level was found to be greatly increased in patients who died compared to those who did not. (8) A similar result was also stated by Ma et al. who found a relationship between high RIPK3 level and severe organ failure. (10) Extracellular RIPK3 level was highest in non-survived patients than survived and discharged groups. (10)

It is important to note a few limitations of this study, which could impact broader applicability of our findings. This study only conducted a one-time examination of RIPK3 level when the diagnosis of sepsis was made. This was a single centered study that the result may not represent other institution's specifications.

Conclusions

This single centered observational study resulted in high 28-day sepsis-related mortality (67.8%). High levels of RIPK3 (≥0.51 ng/ml) were able to predict sepsis-related mortality. We suggest further studies which take our limitations into consideration.

Competing interests

The author(s) declared no potential competing interest with respect to any patents, patent applications, or products in development or for market.

Table 1. Participant's characteristics

Characteristics	Outcomes	
	Non-survivors (n=40)	Survivors (n=19)
Age (years), mean±SD	51.72±14.5	47.36±15.63
Gender		
- Male, n (%)	22 (55.00)	8 (42.11)
- Female, n (%)	18 (45.00)	11 (57.89)
Diagnosis		
- Elective surgery, n (%)	6 (15.00)	0 (0.00)
- Emergency surgery, n (%)	9 (22.50)	8 (42.11)
- Medical, n (%)	25 (62.50)	11 (57.89)
Charlson's comorbidity score		
- Score 0, n (%)	2 (5.00)	3 (15.79)
- Score 1-2, n (%)	28 (70.00)	11 (57.89)
- Score 3-4, n (%)	4 (10.00)	2 (10.53)
- Score ≥5, n (%)	6 (15.00)	3 (15.79)
Albumin level		
- <3.5 g/dl, n (%)	39 (97.50)	17 (89.47)
- ≥3.5 g/dl, n (%)	1 (2.50)	2 (10.53)
SOFA score, mean±SD	10.9±3.38	8.10±3.07
- Score ≤12, n (%)	26 (65.00)	18 (94.74)
- Score >12, n (%)	14 (35.00)	1 (5.26)
APACHE II score, mean±SD	23.3±4.66	16.57±4.13
Infection sources		
- Lungs, n (%)	24 (60.00)	9 (47.37)
- Abdomen, n (%)	8 (20.00)	5 (26.32)
- Skin/soft tissue, n (%)	6 (15.00)	4 (21.05)
- Neurology, n (%)	1 (2.05)	1 (5.26)
- Others	1 (2.50)	0 (0.00)
Microbiology		
Culture results		
- Gram positive, n (%)	3 (7.5)	1 (5.26)
- Gram negative, n (%)	21 (52.50)	14 (73.68)
- Fungi, n (%)	4 (10.00)	2 (10.53)
- Others, n (%)	12 (30.00)	2 (10.53)
Antibiotics sensitivity		
- Non-MDR, n (%)	24 (60.00)	8 (42.11)
- MDR, n (%)	16 (40.00)	11 (57.89)
Secondary infection		
- No, n (%)	28 (70.00)	17 (89.47)
- Yes, n (%)	12 (30.00)	3 (10.53)
Patient admission		
- Non-emergency department, n (%)	10 (25.00)	9 (47.37)
- Emergency department, n (%)	30 (75.00)	10 (52.63)
Lactate level (mmol/l), mean±SD	4.05±3.01	2.65±1.58
RIPK3 level (ng/ml), mean±SD	1.27±0.31	0.43±0.35

Legend: SOFA=sequential (sepsis-related) organ function assessment; APACHE=acute physiology and chronic health evaluation; MDR=multi drug resistance; RIPK3=receptor-interacting protein kinase 3.

Table 2. Area under the ROC curves (AUC) for predicting mortality

Risk factor	AUC	Cutoff value	Sensitivity	Specificity	95% confidence interval	
					Lower	Upper
RIPK3	0.925	0.51	92.5	89.5	0.826	0.977

Legend: ROC=receiver operating characteristic; RIPK3=receptor-interacting protein kinase 3.

Table 3. Bivariate analysis

Risk factor	Outcomes		RR	95% confidence interval		p
	Non-survivors n (%)	Survivors n (%)		Lower	Upper	
RIPK3 level ≥0.51 ng/ml	37 (92.50%)	3 (7.50%)	5.86	2.066	16.610	<0.0001
RIPK3 level <0.51 ng/ml	3 (15.79%)	16 (84.21%)				

Legend: RIPK3=receptor-interacting protein kinase 3; RR=relative risks.

Figure 1. Participants recruitment chart

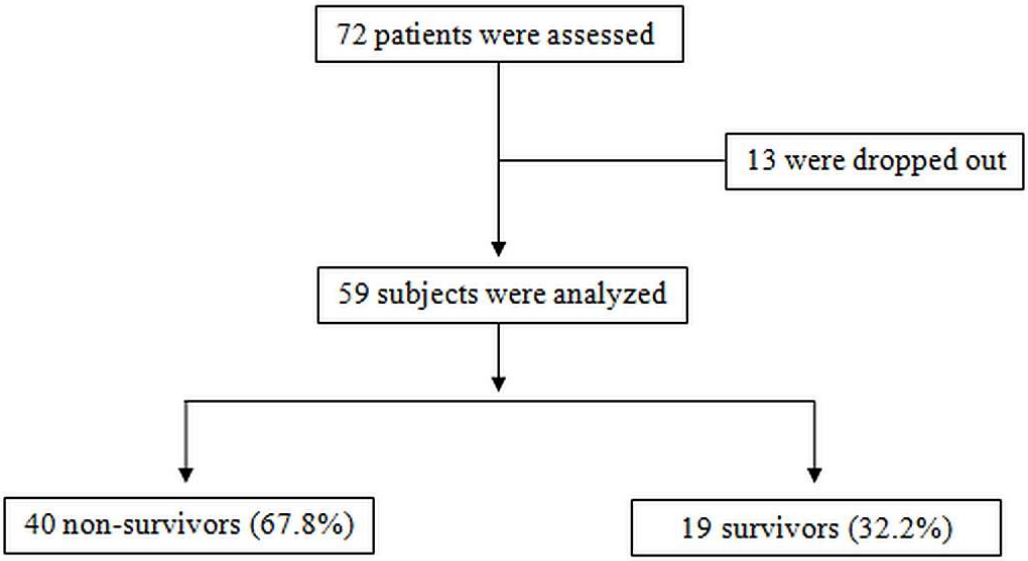
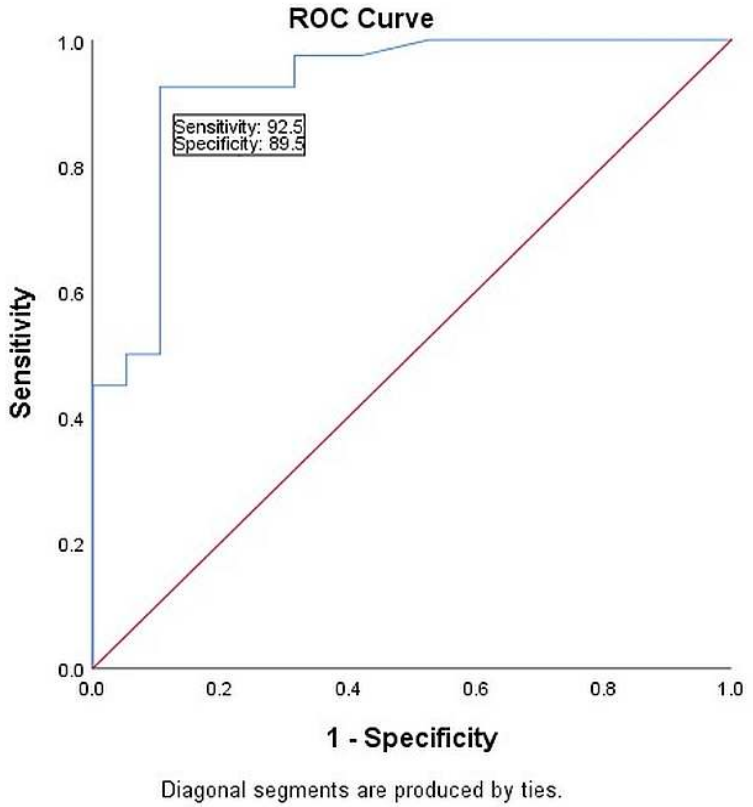


Figure 2. ROC curve



Legend: ROC=receiver operating characteristic.

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