Persistent Lymphopenia as a Predictor of In-Hospitaliy in Septic Patients at Dr. Sardjito Hospital

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Abstract

Background: Sepsis, a life-threatening organ dysfunction caused by deregulation of body response to an infection with a high mortality rate. Pro-inflammatory cytokines are related to early mortality related sepsis, and immune dysfunction and suppression characterized by lymphocyte loss are related to late mortality. Persistent lymphopenia is a good biomarker for immunosuppression and predicts mortality in sepsis patients. Lymphocyte counts are easily measured and cheaper than other inflammation marker for sepsis.
Aims: The objective of this study was to determine whether persistent lymphopenia has a predictive value for mortality in septic patients at Dr. Sardjito General Hospital.
Methods: This study was a retrospective cohort study, sepsis and lymphogenic patients admitted to Internal Medicine ward between January 1, 2016 and December 31, 2017. Lymphocytes were count at day 1 and 4 following the diagnosis of sepsis. Persistent lymphopenia was defined as an absolute lymphocyte count of $1.62x10^3/\mu L$ or less on day 4. The primary outcome was mortality at the end of hospitalization.
Results: 126 adult patients, 101 with persistent lymphopenia, 25 non-persistent lymphopenia, 47 patients died (37.3%). Patients with persistent lymphopenia significantly at risk of death (P=0.003, OR 5.66, 95% CI 1.59-20.13) than non-persistent lymphopenia. Logistic regression was used to account for potential confounding factors, persistent lymphopenia (p = 0.003, OR 8.01, 95% CI 2.04-31.45) and skin and soft tissue infection (p = 0.017, OR 2.94, 95% CI 1.21-7.14) were significantly associated with mortality in sepsis patients at Dr. Sardjito General Hospital.
Conclusion: Persistent lymphopenia predicts mortality in adult patients with sepsis at Dr. Sardjito General Hospital.

Keywords: *Persistent lymphopenia, Predictor of mortality, Sepsis.*

Abstrak

Latar Belakang*. Sepsis, disfungsi organ yang mengancam jiwa yang disebabkan oleh deregulasi respons tubuh terhadap infeksi dengan tingkat kematian yang tinggi. Sitokin pro-inflamasi terkait dengan sepsis terkait kematian dini, dan disfungsi imun dan penekanan yang ditandai dengan hilangnya limfosit terkait dengan kematian lanjut. Limfopenia persisten adalah biomarker yang baik untuk imunosupresi dan memprediksi mortalitas pada pasien sepsis. Jumlah limfosit mudah diukur dan lebih murah daripada penanda peradangan lainnya untuk sepsis.*
Tujuan. *Tujuan dari penelitian ini adalah untuk menentukan apakah limfopenia persisten memiliki nilai prediktif untuk mortalitas pada pasien septik di Rumah Sakit Dr. Sardjito.*
Metode. *Ini adalah studi kohort retrospektif pada pasien sepsis dan limfopenik yang dirawat di bangsal Penyakit Dalam antara 1 Januari 2016 dan 31 Desember 2017. Limfosit dihitung pada hari 1 dan 4 setelah diagnosis sepsis. Limfopenia persisten didefinisikan sebagai jumlah limfosit absolut* $1.62x10^3/\mu L$ *atau kurang pada hari ke-4. Hasil utamanya adalah kematian pada akhir rawat inap.*

Hasil. *Penelitian ini terdiri dari 126 pasien dewasa terdiri dari 101 pasien dengan limfopenia persisten dan 25 limfopenia non-persisten. Sebanyak 47 pasien meninggal (37,3%). Pasien dengan limfopenia persisten secara signifikan berisiko kematian (P = 0,003, OR 5,66, 95% CI 1,59-20,13) daripada limfopenia non-persisten. Regresi logistik digunakan untuk memperhitungkan faktor pembaur potensial, limfopenia persisten (p = 0,003, OR 8,01, 95% CI 2,04-31,45) dan infeksi kulit dan jaringan lunak (p = 0,017, OR 2,94, 95% CI 1,21-7,14) secara signifikan berhubungan dengan kematian pada pasien sepsis di RSUP Dr. Sardjito.*
Kesimpulan. *Limfopenia persisten memprediksi kematian pada pasien dewasa dengan sepsis di Rumah Sakit Dr. Sardjito.*

Kata kunci: *sirosis hati, simvastatin, fibroscan, transient elastography*

Introduction

Sepsis, a life-threatening organ dysfunction caused by dysregulation of the body's response to infection, is one of the longest-standing and most difficult syndromes in medicine.[1] Recent epidemiological studies have shown that sepsis remains a large and the severe health burden throughout the world.[2] Data from the Dr. Sardjito General Hospital Medical Record Installation in Yogyakarta (unpublished data) shows the average mortality rate of Internal Medicine Unit patients with sepsis in 3 years (2015-2017) reached 60%.

In the sepsis consensus published in 2015, it was recommended to use a change in total Sequential (Sepsis-Related) Organ Failure Assessment (SOFA) scores to assess organ dysfunction. SOFA score ≥ 2 points have an increased risk of mortality reaching 2-25 times. These score components require laboratory tests such as bilirubin, creatinine, and blood gas analysis.[1] While some regions in Indonesia with inadequate facilities and infrastructure require a simpler and cheaper examination, such as lymphocyte counts.

Significant decrease of lymphocytes occurs through the process of apoptosis, which is an important factor in the occurrence of immunosuppression conditions at an advanced stage of sepsis, which makes patients prone to new infections and death.[4] Some studies have proven that circulating lymphocyte levels will decrease at onset of sepsis and can continue to fall until the 28th day. Persistence of lymphopenia is also associated with a poor prognosis. Vulliamy et al.[16], proved that surgical patients with critical conditions and had persistent lymphopenia until the 7th day of treatment at the ICU had a 3.5-fold risk of mortality. Research by Drewry et al.[6] showed persistent lymphopenia on day 4 after onset of sepsis being a predictor factor for survival in days 28 and 1 year. Lymphocyte counts are easy to measure and inexpensive compared to other inflammatory markers. For this reason, a study is needed to determine whether persistent lymphopenia has a predictive value for the mortality of patients with sepsis who are treated in internal medicine units at Dr. Sardjito General Hospital. The difference with the research that has been done is that the subjects in this study are sepsis patients treated in internal medicine units only, where as far as researchers are aware, subjects in previous studies include patients from various treatment units, and are conducted abroad, especially in western countries.

Methods

This study was a retrospective cohort study. This research was conducted at the Medical Record Installation of Dr. Sardjito General Hospital in Yogyakarta. The source population of the study was septic patients treated by the Internal Medicine Unit team of Dr. Sardjito General Hospital in the period January 2016-December 2017. Subjects were septic patients who were first diagnosed either when they first came to the Emergency Room (ER) or were being treated in the Internal Medicine ward.

The inclusion criteria were 18-years-old adult patients who were diagnosed with sepsis and lymphopenia, treated in the emergency room, inpatient in internal medicine wards and had data of lymphocyte counts on day-1 and day-4. Operational definitions for sepsis here is people who meet SSC-SIRS and Sepsis-3 criteria. Exclusion criteria included concomitant HIV, malignancies, autoimmune diseases, history of G-CSF preparations used in the past 1 month, as well as history of steroid and/or immunosuppressant used for more than 2 weeks in the last 1 month.

Lymphocyte counts were obtained from two examinations during the hospitalization period, with the first examination carried out when the diagnosis of sepsis was established (D-1 lymphocytes), and the second examination within 48-96 hours after the first examination (D-4 lymphocytes). Lymphopenia was the total count of lymphocytes below the normal value that the patient has. The normal value based on hematology examination tools used in Dr. Sardjito General Hospital Yogyakarta was 1.62-5.37 x 10^3/L. Persistent lymphopenia was a constant low number of D-4 lymphocytes below normal value. Age is is calculated from the date of birth (in years). Body Mass Index) values are calculated based on height and weight. Malnutrition is defined as Nutritional status based on BMI value <18.5 kg / m2. Hypoalbuminemia is defined as Albumin <3.4 g / dL. Multiple infection defined as More than 1 infection obtained at the same time.

The independent variable was the persistence of lymphopenia which occurs compared to the baseline when sepsis was diagnosed. The dependent variable in this study was mortality. Bivariate statistical analysis performed with the Chi-Square test and calculating OR (Odds Ratio) to determine the magnitude of risk among septic patients with persistent and non-persistent lymphopenia. It was said to be statistically significant if p <0.05 with a 95% confidence interval and OR> 1. Then a multivariate analysis test was performed with Logistic Regression to assess which variable was more statistically significant. This study used the approval of the Faculty of Medicine, Public Health and Nursing (FKKMK) biomedical research ethics commission at the Gadjah Mada University Yogyakarta as well as permission from the Director of the Dr. Sardjito General Hospital Yogyakarta with ref number: KE/FK/0443/EC/2018.

Results

A total of 126 subjects met the inclusion and exclusion criteria (figure 1), Subjects were divided based on persistency of lymphopenia, 101 subjects with persistent lymphopenia and 25 subjects with non-persistent lymphopenia.

Sociodemographic and laboratory characteristics shown in table 1. The number of male study subjects was 64.3%. The mean age was 55 (± 15.36) years, with 28.6% of subjects aged ≥ 65 years. Only 109 (86.5%) subjects

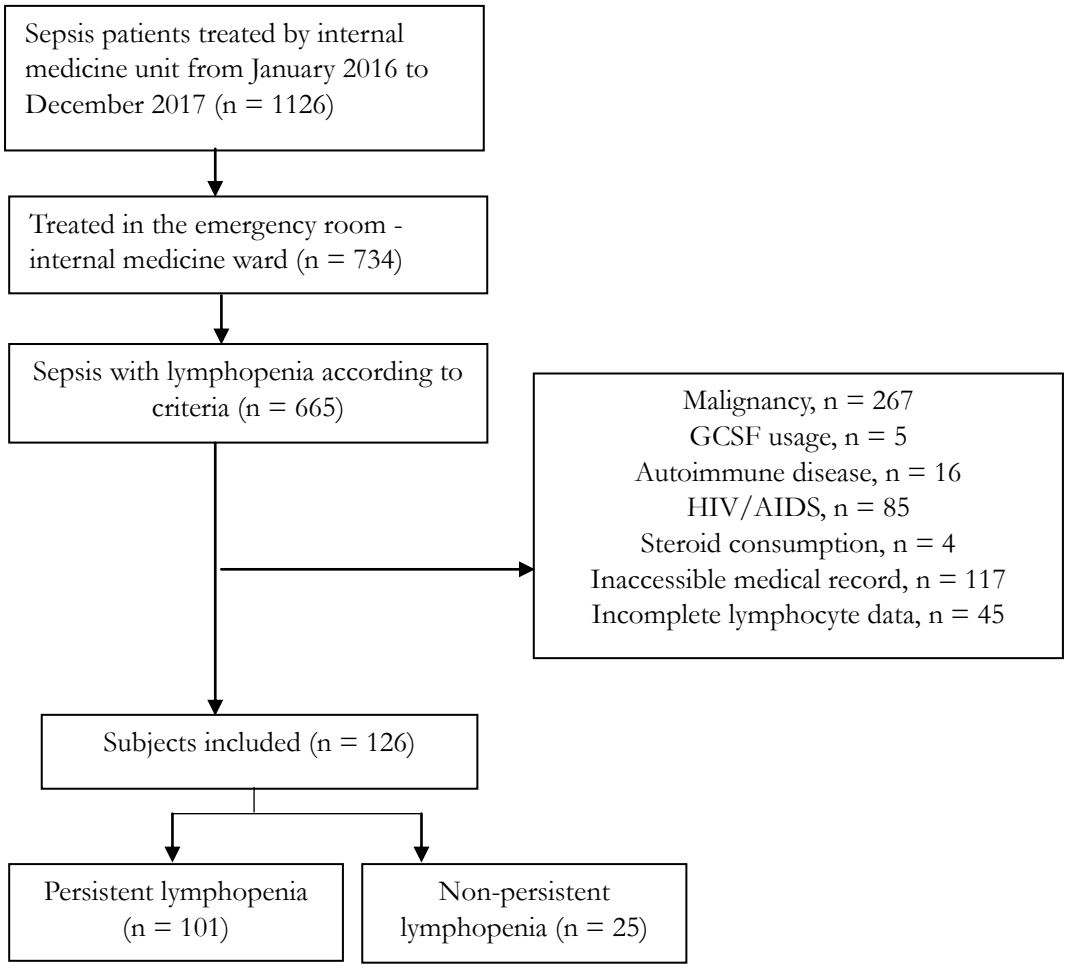

Figure 1. Flowchart of inclusion and exclusion of research subjects

had culture data and material sensitivity from the source of infection, the remaining 13.5% did not have culture data (table 1). The main sources of infection were lung infections (70.6%), followed by urinary tract infection by 32.5%, and infections of the skin and soft tissues by 28.6%. A total of 37 subjects (29.4%) experienced septic shock or received vasopressors. Death occurred in 47 subjects (37.3%).

There were no differences in the characteristics of the subjects in the persistent lymphopenia and non-persistent lymphopenia groups (Table 2). But there were significant differences between the values of D-1, D-4 lymphocytes, and the mean difference in D-1 and D-4 lymphocytes in both groups, where lymphocyte counts were lower in the persistent lymphopenia group.

Comparison of lymphocyte counts based on outcome showed that there was no significant difference in the mean value of D-1 lymphocytes (table 3). However, there were significant differences in the mean value of D-4 lymphocytes and the difference in lymphocyte mean between the two groups, where the increase in the average number of lymphocytes in living subjects reached 396.89 $\pm$ 675.99/µL while in subjects who died, lymphocytes only increased amounting to 56.84 $\pm$ 418.89/µL.

Table 1. Sociodemographic and Laboratory Characteristics of Septic Patients with Lymphopenia

Subject Characteristics	Total (N=126)	Mean (SD)	Median
Gender (n (%))			
- Male	81 (64.3)		
- Female	45 (35.7)		
Age (years old) (Mean ±SD)		55.09 (15.36)	
- < 65 years (%)	90 (71.4)		
- ≥ 65 years n (%	36 (28.6)		
Nutritional status			
- BMI < 18.5 kg/m^2 (%)	35 (27.8)		
- BMI >= 18.5 kg/m^2 (%)	91 (72.2)		
Lymphocyte D-1 (/µL)		859.81 (± 368.21)	860.22 (172.1 – 1560.78)
Lymphocyte D-4 (/µL)		1129.86 (± 648.40)	1041.35 (137.69 – 3416.49)
Albumin level (gr/dL)		2.81 (± 0.71)	
Albumin level category			
- Low (<3.4 gr/dL)	100 (79.4)		
- Normal (≥ 3.4 gr/dL)	26 (20.6)		
Culture data result (%)			
- Available	109 (86.5)		
- Not available	17 (13.5)		
Culture *			
Positive culture result (%)	75 (68.8)		
- Gram positive	41 (37.6)		
- Gram negative	47 (43.1)		
- Fungal	21 (19.3)		
- Polymicrobial (>1)	30 (27.5)		
Negative culture result	34 (31.2)		
Lymphopenia (n(%)			
- Persistent	101 (80.2)		
- Non Persistent	25 (19.8)		
Combination antibiotics (n(%))	109 (86.5)		
Diagnosis criteria (n (%))			
-SIRS	89 (70.7)		
-SOFA	12 (9.5)		
-SIRS + SOFA	25 (19.8)		

Source of infection** n (%)		
- Lung	89 (70.6)	
- Urinary tract	41 (32.5)	
- Abdomen	16 (12.7)	
- Skin-soft tissue	36 (28.6)	
- Others	12 (9.5)	
Multiple sources of infection (>1)	58 (46)	
Comorbid** n(%)		
- DM	45 (35.7)	
- CKD	30 (23.8)	
- Stroke	7 (5.6)	
- Cirrhosis hepatic	9 (7.1)	
- Heart Failure	19 (15.1)	
- COPD	13 (10.3)	
- Hypertension	32 (25.4)	
- Depression	8 (6.4)	
Multiple comorbidities (>= 2) n (%)	45 (36.5)	
Shock n (%)	37(29.4)	
Length of stay (days)		12.61 (6.60)
Outcome (n(%))		
- Alive	79 (62.7)	
- Dead	47 (37.3)	

*Percentage based on the amount of culture data available
** Percentage> 100% because there were more than 1 event in 1 subject

Table 3. Lymphocyte Counts based on Mortality Outcome in Septic Patients

Value	Alive (N=79)	Dead (N=47)	p
Lymphocyte D-1 (/µL) (Mean± SD)	854.21 ± 389.06	860.21 ± 334.07	0.826*
Lymphocyte D-4(/µL) (Mean± SD)	1251.11 ± 720.60	926.05 ± 440.75	0.021**
Mean Difference of Lymphocyte D-4 and D-1	396.89 ± 675.99	56.84 ± 418.89	0.012**

* *Independent t-test,* ** Mann-Whitney test

Table 2. Characteristics of Septic Patients based on persistent Lymphopenia

Characteristic	Persistent Limphopenia		Non Persistent Limphopenia		p
	(N=101)	N %	(N=25)	N %	
Sex					
Female	35	34.7	10	40.0	0.617*
Male	66	65.3	15	60.0	
Age			21		
< 65 years	69	68.3	4	84.0	0.120*
65 years	32	31.7		16.0	
Nutritional Status					
Malnutrition	31	30.7	4	16.0	0.142*
Normal	70	69.3	21	84.0	
Albumin Level					
Normal	22	21.8	4	16.0	0.522*
Hypoalbuminemia	79	78.2	21	84.0	
Infection source					
Lung	75	74.3	14	56.0	0.073*
Urinarius Tract	35	34.7	6	24.0	0.309*
Abdomen	14	13.9	2	8.0	0.342*
Skin-soft tissue	28	27.7	8	32.0	0.672*
Others	8	7.9	4	16.0	0.192*
Multiple Infection	49	48.5	9	36.0	0.261*
(>1) Comorbid Diabetes	33	32.7	12	48.0	0.152*
CKD	24	23.8	6	24.0	0.98*
Stroke	6	5.9	1	4.0	0.579**
Cirrhosis Hepatis	8	7.9	1	4.0	0.434**
CHF	16	15.8	3	12.0	0.451**
COPD	13	12.9	0	0	0.048**
Hypertension	27	26.7	5	20.0	0.489*
Depression	8	7.9	0	0	0.161**
Multiple Comorbid (≥2)	36	35.6	9	36.0	0.973*
Antibiotic combination	88	87.1	21	84.0	0.477**
Shock	30	29.7	7	28.0	0.867*
Length of stay (Days)					0149***
Means ± SD	13.06	± 6.77	10.8	4 ± 5.66	
Lymphocyte D-1 (/µL) (Mean± SD) (Mean± SD)	802.11 ± 359.89		1092.92 ± 309.59		<0.001***
Lymphocyte D-4(/µL) (Mean± SD)	875.74 ± 356.38		2156,48 ± 539,31		<0,001***
Mean Difference of Lymphocyte D-4 and D-1	73.63 ± 426.86		1063,56 ± 621,27		<0,001***

*Chi-square ** Fisher's exact test *** Mann-Whitney test

Bivariate analysis (table 4) shown the predictor factors that have a significant relationship with mortality were persistent lymphopenia (p= 0.003), multiple infections (p= 0.047), and skin and soft tissue infections (p= 0.007). Septic patients with persistent lymphopenia at risk of death 5.66 times (95% CI 1.59-20.13) more than persistent non-lymphopenia.

Table 4. Bivariate Analysis of Septic Patients with Lymphopenia on Mortality

Predictor	Alive (n=79)	Dead (n=47)	p	OR	95% CI	
					Lower	Upper
Persistent lymphopenia	58 (73.4%)	44 (93.6%)	0.003*	5.66	1.59	20.13
Gender (Male)	51 (64.5%)	30 (63.8%)	0.934	0.96	0.45	2.05
Age >= 65 years	19 (52.8%)	17 (36.2%)	0.145	1.78	0.81	3.93
Malnutrition	18 (22.8%)	17 (36.2%)	0.105	1.92	0.86	4.24
Hypoalbuminemia	59 (74.7%)	41 (87.2%)	0.092	2.31	0.85	6.26
Source of infection						
- Tr. respiratorius	53 (67.1%)	36 (76.6%)	0.257	1.60	0.70	3.65
- Tr. urinarius	23 (29.1%)	18 (38.3%)	0.287	1.51	0.70	3.24
- Abdomen	9 (11.4%)	7 (14.9%)	0.568	1.36	0.47	3.93
- Skin and soft tissue	16 (20.3%)	20 (42.5%)	0.007*	2.91	1.31	6.47
- Other infection	10 (12.7%)	2 (4.3%)	0.12	0.30	0.06	1.46
Multiple infections	31 (39.2%)	27 (57.4%)	0.047*	2.09	1.00	4.35
Comorbidity						
- DM	24 (30.4%)	21 (44.7%)	0.105	1.85	0.87	3.91
- CKD	20 (25.3%)	10 (21.2%)	0.607	0.79	0.33	1.89
- Stroke	2 (2.5%)	5 (10.6%)	0.055	4.58	0.85	24.65
- Cirrhosis hepatic	6 (7.6%)	3 (6.4%)	0.798	0.83	0.19	3.48
- Heart Failure	14 (17.7%)	5 (10.6%)	0.283	0.55	0.18	1.64
- COPD	8 (10.1%)	5 (10.6%)	0.927	1.05	0.32	3.44
- Hypertension	18 (22.8%)	14 (29.8%)	0.383	1.43	0.63	3.25
- Depression	4 (5%)	4 (8.6%)	0.443	1.74	0.41	7.33
Multiple comorbidities	28 (35.4%)	17 (36.2%)	0.934	1.03	0.48	2.19
Combination antibiotic	67 (84.8%)	42 (89.4%)	0.47	1.50	0.49	4.57
Shock	19 (24%)	18 (38.3%)	0.089	1.96	0.89	4.28

* P value <0.05 = statistically significant

Independent sample t-test for duration of hospitalization displayed no significant results (p = 0.998). Multivariate analysis was performed to control confounding factors. The final model of multivariate analysis was shown in Table 5, showed significant independent predictors for mortality in sepsis from this study were persistent lymphopenia, skin, and soft tissue infections.

Due to the retrospective method, there was limitation with completeness of data, consequently there were 17 subjects without culture result (table 1). Meanwhile from a prospective cohort study by Asdie et al. (2017)[10], it was known that positive blood

Table 5. Multivariate Logistic Regression Analysis of Septic Patients with Lymphopenia on Mortality Outcome

Variable	OR	95% CI	p
Model 5 Lymphopenia status			
Non-Persistent lymphopenia	Ref.	-	-
Persistent lymphopenia	8.01	2.04-31.45	0.003*
Source of infection			
Without infection	Ref.	-	-
Skin and soft tissue infection	2.94	1.21-7.14	0.017*
Comorbidity			
Without comorbidity	Ref.	-	-
DM	1.82	0.77-4.27	0.170
Constanta	0.058	-	0.000

*P value <0.05 = statistically significant (N=126)

Table 6. Bivariate Analysis of Septic Patients with Lymphopenia that have Culture Data on Mortality

Predictor	Alive (n=68)	Dead (n=41)	p	OR	95% CI	
					lower	upper
Persistent lymphopenia	51 (75%)	41 (87.2%)	0.021*	4.22	1.15	15.45
gender (male)	42 (61.8%)	27 (65.9%)	0.668	1.19	0.53	2.68
Age >= 65 years	19 (27.9%)	14 (34.1%)	0.495	1.33	0.58	3.08
Malnutrition	16 (23.6%)	15 (36.6%)	0.143	1.87	0.80	4.37
Hypoalbuminemia	51 (75%)	36 (87.8%)	0.107	2.4	0.81	7.1
Source of infection						
- Tr. respiratorius	46 (67.6%)	31 (75.6%)	0.377	1.48	0.61	3.55
- Tr. urinarius	23 (33.8%)	15 (36.6%)	0.769	1.12	0.502	2.53
- Abdomen	8 (11.8%)	5 (12.2%)	0.946	1.04	0.31	3.42
- Skin and soft tissue	14 (20.6%)	20 (48.8%)	0.002*	3.67	1.57	8.58
- Other infection	7 (10.3%)	1 (2.4%)	0.128	0.21	0.02	1.83
Multiple infections	29 (42.6%)	24 (58.8%)	0.108	1.89	0.86	4.16
Comorbidity						
- DM	21 (30.8%)	19 (46.3%)	0.105	1.93	0.86	4.30
- CKD	18 (26.5%)	9 (22%)	0.596	0.78	0.31	1.95
- Stroke	2 (2.9%)	4 (9.8%)	0.131	3.56	0.62	20.41
- Cirrhosis hepatic	4 (5.9%)	3 (7.3%)	0.767	1.26	0.26	5.95
- Heart Failure	12 (17.6%)	4 (9.8%)	0.259	0.50	0.15	1.68
- COPD	7 (10.3%)	5 (12.2%)	0.759	1.21	0.35	4.09
- Hypertension	16 (23.5%)	13 (31.7%)	0.349	1.50	0.63	3.58
- Depression	4 (5.9%)	4 (9.8%)	0.453	1.73	0.40	7.32
Multiple comorbidities	24 (35.3%)	16 (39%)	0.695	1.17	0.52	2.61
Combination antibiotic	60 (88.2%)	37 (90.2%)	0.746	1.23	0.34	4.38
Shock	15 (22%)	17 (41.5%)	0.031*	2.50	1.07	5.82
Positive culture	44 (64.7%)	31 (75.6%)	0.234	1.69	0.70	4.03
Gram positive pathogen	25 (36.8%)	16 (39%)	0.813	1.10	0.49	2.44
Gram negative pathogen	26 (38.2%)	21 (51.2%)	0.185	1.69	0.77	3.71
Fungal pathogen	15 (22%)	6 (14.6%)	0.341	0.60	0.21	1.71
Polymicrobial	21 (30.8%)	9 (22%)	0.312	0.62	0.25	1.55

* P value <0.05 = statistically significant (N=109)

culture results could be used to predict the severity of sepsis and the incidence of death due to sepsis. To find out whether culture growth and type of pathogen were predictor factor of death in this study, and avoiding bias by excluding subjects without culture data, an additional analysis was carried out on a subgroup of research subjects who had culture result (n = 109).

Bivariate analysis (table 6) displayed a slight difference in result for this subgroup. First, the condition of shock was a significant predictor of death (p= 0.031, OR 2.50, 95% CI 1.07-5.82). Second, multiple infection was not a significant predictor (p = 0.108). Meanwhile, persistent lymphopenia (p= 0.021, OR 4.22, 95% CI 1.15-15.45) and skin and soft tissue infections (p= 0.002, OR 3.67, 95% CI 1.57- 8.58) remains a significant predictor of death in sepsis. Independent sample t-test for duration of hospitalization showed no significant results (p = 0.734).

Multivariate logistic regression analysis in this sub-group was seen in table 7. Statistically significant independent predictor factors for death were persistent lymphopenia (p = 0.011,

OR 6.04, CI 1.5-24.3) and skin infections and soft tissue (p = 0.001, OR 4.68, CI 1.86-11.78). These results were the same as the analysis of the entire research subject group.

Table 7. Multivariate Logistic Regression Analysis of Septic Patients with Lymphopenia that Have Culture Data on Mortality

Variable	OR	95% CI	P
Model 5 Lymphopenia status			
Non-Persistent lymphopenia	Ref.	-	-
Persistent lymphopenia	6.04	1.50-24.30	0.011*
Source of infection			
Without infection	Ref.	-	-
Skin and soft tissue infection	4.68	1.86-11.8	0.001*
Constanta	0.078	-	0.000

*P value <0.05 = statistically significant (N=109)

Discussion

In this study, male subjects were found as much as 64.3%. The mean age was 55 (± 15.36) years, with 28.6% of subjects aged ≥ 65 years. Age and sex as risk factors for sepsis and mortality have been known from various studies. Research by Fleischmann, et al. (2016)[2] showed a higher incidence of sepsis in men up to 1.8 times that of women, and mortality increased with increasing age over 40 years. Hypoalbuminemia occurred in 79.4% (100 subjects) with an average albumin level of 2.81 (± 0.7) g/dL. In sepsis, endothelial damage increases trans-capillary albumin loss, reaching 13 times the normal level.[6]

The main source of infections was infection of the lungs, followed by infection of the urinary tract and infections of skin and soft tissue. This was different from other studies, where the main sources of sepsis infection were lung infection, urinary tract and intra-abdomen infection.[7] The administration of combination antibiotics occurred in 109 subjects (86.5%) with the first empiric antibiotics most given were the cephalosporins (86.5%), combined with the quinolone group (50.4%) (data not shown). Further analysis based on the type of antibiotic was not carried out related to the uniformity of the antibiotic classes given.

The mortality rate in this study reached 37.3%, consistent with previous studies, where mortality in sepsis was 20-30%, and more severe in septic shock, at 58.8% .[2,9] The results of the bivariate analysis showed that predictor factors significantly related with death were persistent lymphopenia (p = 0.003), skin and soft tissue infections (p = 0.007), and multiple sources of infection (p = 0.047). The results of this study are consistent with previous studies related to persistent lymphopenia in sepsis.[4,5,11] Multivariate analysis also supports that significant independent predictors for mortality in sepsis from this study were persistent lymphopenia (p = 0.003, OR 8.01, 95% CI 2.04-31.45) and skin and soft tissue infections (p = 0.017, OR 2.94, 95% CI 1.21-7.14).

The accumulation of pro-inflammatory cytokines was associated with death in the early phase of sepsis. While deaths due to sepsis in the later stages were related to conditions of suppression and immune dysfunction. Research that has been done shown that most patients who passed through the early phase of sepsis will enter an immunological phase that was unresponsive (unresponsive state), which characterized by a decreased in T cell function, indicated the condition of immunosuppression or immune paralysis. Lymphocyte loss was a major feature of immune suppression in critically ill patients. In fact, more than 70% of deaths due to sepsis occur within the first 3 days, with subsequent deaths occurring within weeks after the onset of sepsis.[12] In this study, deaths occurred in 37.3% of subjects with a

mean length of stay until the death occurred was 12.6 (± 7.9) days. This was consistent with death that occured in the advanced stages of sepsis related to immunosuppression and associated with persistent lymphopenia.

Previous observational studies have shown an association between lymphopenia related to sepsis and death. For example, Chung et al. (2015)[13] observed the presence of severe lymphopenia in septic shock patients at the admission of ICU associated with a 3.5-fold increase in mortality on the 28th day. Research by Drewry et al. (2014)[5] in tertiary education-based hospitals in the US showed persistent lymphopenia on day 4 after onset of sepsis being a predictor factor for survival in days 28 and 1 year. As many as 22% of patients died on day 28, and severe persistent lymphopenia (<0.6 x 10^3/L) was associated with increased secondary infections. This study observed persistent lymphopenia in sepsis patients treated in the internal medicine ward and found an 8-fold increased risk of death at the end of hospitalization.

Diabetes mellitus (DM) is the most common comorbid in this study but after being analyzed statistically there was no significant relationship with mortality. Esper, et al. (2009)[14] also found the same thing, this was related to the theory that increased leptin in patients with type 2 DM has protective properties against inflammation in sepsis. On the other hand, DM as the most common comorbid in this study related to skin and soft tissue infections became the 3rd most source of infection (28.5%). The most common types of skin and soft tissue infections in this study were DM ulcers, as many as 17 of 36 subjects. Another caused for multitude cases can be attributed to the role of Dr. Sardjito General Hospital as the highest-level referral hospital, so that the type of infections treated was a severe one. Research by Lipsky, et al. (2010)[15] found independent risk factors for death in skin and soft tissue infections cases with DM are the types of infections, namely DM ulcers and surgical scars and the severity of the disease.

From the available culture data, pathogen growth was 68.8%. The main pathogens were gram negative (43.1%), followed by gram positive 37.6% and fungi as much as 19.3%, with polymicrobial infections (pathogen > 1 species) occurring as much as 27.5%. This was not different from other studies, where gram negative was the main cause of sepsis.[8] Negative culture results were obtained at 31.2%, according to Angus and van der Poll's research (2013)[9], where one third of cultures from all sources of infection did not showed germ growth.

In this study, both gram-negative and polymicrobial pathogens, and the presence of pathogen growth, were not a risk factor for death. Previous studies have shown the type of pathogen was related to mortality. According to Polat (2017)[8], the mortality rate in sepsis due to gram negative reaches 45-50%, gram positive by 20-30%, and anaerobic bacteria by 15-30%. Research by Asdie et al. (2017)[10] also showed more deaths in sepsis with gram negative pathogenic blood culture results, and positive blood cultures were predictors of death in septic patients, associated with high bacterial load. Lipsky, et al. (2010)[15] found in DM subjects with skin and soft tissue infections, the type of pathogen that affects death is a single gram-negative and polymicrobial pathogen that includes P. aeruginosa species. The difference in these results can be caused because this study did not analyze the type of pathogenic species or the tissue/organ sources of culture.

This study has several limitations. The retrospective cohort design caused the time for blood D-4 lymphocytes examination not be uniform in all study subjects, so it was based on the timeframe 48-96 hours after examination of D-1 lymphocytes. The management of sepsis was only based on recorded data and assumed to be in accordance with the SSC protocol, thus allowing a bias in the outcome. Further analysis of the confounding variables of the causative pathogenic species and the type of antibiotic was not carried out. Further research with a prospective design was needed to control outcome bias and can be followed by survival analysis and validation of sepsis prognosis scores. Research that includes analysis of confounding variables of causative pathogenic species and types of antibiotics as well as analysis of lymphocyte subset cells affected in sepsis is also needed, so it could be used for further research related to therapy in sepsis.

Conclusion

Persistent lymphopenia could be used as a predictor of mortality in adult patients with sepsis at Dr. Sardjito General Hospital, with the risk of death reaching an 8-fold increased at the end of hospitalization.

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