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Persistent lymphopenia in septic patients at Dr. Sardjito General Hospital, Yogyakarta

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ABSTRACT

Sepsis triggers immune responseboth pro-inflammatory and anti-inflammatory. Lymphocytes play an important role in the regulation of the inflammatory response. The decrease in lymphocyte numbers due to continuous apoptosis by sepsiscan suppress the immune system and failure to resolve inflammation. Persistent lymphopenia is also associated with a poor prognosis of sepsis. Currently, there are limited studies about persistent lymphopenia in sepsis patients in low- and middle-income countries, including Indonesia. This study aimed to describe the sociodemographic, clinical, and laboratory patterns of sepsis patients with persistent lymphopenia. This was a descriptive study that analyzed patients' medical records who were treated at the Department of Internal Medicine, Dr. Sardjito General Hospital, Yogyakarta from January 1st, 2016, to December 31th, 2017. Patients diagnosed with clinical sepsis and persistent lymphopenia were included in the study. The status of persistent lymphopenia was described as lymphocyte counts that remained low or lower (<1.62x10^3/L) on day 4± 24 h compared to the initial value at the time of sepsis diagnosis (day one). Information of patients' individual and clinical characteristics, complete blood cell count profiles and culture results were included. The outcomes of interest were the survival status and length of stay of the patients. A total of 101 patients with sepsis and persistent lymphopenia were involved in this study. The average increase in lymphocyte numbers was 73.63 ± 426.86/μL. The main source of infection was pulmonary infection, with a mortality rate of 43.6% and a median survival of 19 days. The average length of stay was 13.1±6.8. Persistent lymphopenia in patients with sepsis has a high mortality. Further research is needed to determine the clinical ramifications of persistent lymphopenia.

ABSTRAK

Sepsis memicu timbulnya mekanisme imun yang bersifat pro-inflamasi dan anti-inflamasi. Limfosit memerankan peranan penting dalam regulasi respon inflamasi, dan menurunnya jumlah limfosit karena apoptosis berkelanjutan akibat sepsis mengarahkan pada supresi system imun dan gagalnya resolusi dari inflamasi. Persistensi limfopenia juga dihubungkan dengan prognosis yang buruk. Sampai saat ini, penelitian terkait limfopenia persisten pada pasien sepsis di negara berkembang termasuk di Indonesia masih terbatas. Penelitian ini bertujuan untuk menggambarkan pola sosiodemografis, klinis, dan laboratoris pasien sepsis dengan limfopenia persisten. Penelitian ini adalah penelitian deskriptif dengan menganalisa rekam medis pasien yang dirawat di Departemen Ilmu Penyakit Dalam, RSUP DR. Sardjito antara 1 Januari 2016 sampai 31 Desember 2017. Pasien dengan klinis sepsis dan limfopenia persisen dilibatkan dalam penelitian ini. Kondisi limfopenia persisten adalah angka limfosit yang tetap rendah atau lebih rendah (<1,62x10^3/μL) pada hari ke-4 ±24 jam disbanding nilai awal saat diagnosis sepsis (harike-satu). Data lain yang disertakan dalam penelitian adalah karakteristik individual dan klinis, profil laboratorium, dan hasil kultur. Luaran yang dinilai adalah *survival* dan lama perawatan. Sebanyak 101 pasien sepsis dengan limfopenia persisten disertakan dalam penelitian ini. Didapatkan peningkatan rerata angka limfosit sebesar 73,63 ± 426,86/μL. Pada pasien dengan limfopenia persistent, didapatkan sumber infeksi utama adalah infeksi paru dengan tingkat mortalitas sebesar 43,6%, dengan median *survival* mencapai 19 hari. Rerata lama perawatan adalah 13,1 ±6,8 hari. Limfopenia persisten pada pasien sepsis memiliki mortalitas tinggi. Perlu dilakukan penelitian lebih lanjut untuk mengetahui makna klinis limfopenia persisten.

Keywords:
persistent lymphopenia;
sepsis;
mortality;
survival rate;
risk factors;

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INTRODUCTION

Sepsis triggers the emergence ofboth pro- and anti-inflammatory immune mechanisms. In general, pro-inflammatory reactions aiming at eradicating invading pathogens are considered responsible for tissue damage in severe sepsis. Where as anti-inflammatory responses that are important to limit local and systemic tissue damage are responsible for the increased susceptibility of the host to secondary infections.[1] Lymphocytes play an important role in the regulation of the inflammatory response. The decrease in the number of lymphocytes due to continuous apoptosis by sepsis leads to suppression of the immune system and failure in controlling inflammation.[2]

A study reported that low lymphocyte counts have better predictive values for bacteremia in patients in the emergency unit (ER) compared to C-reactive protein (CRP) values, leukocyte or neutrophil counts.[3] Furthermore, several studies showed that circulating lymphocyte levels will decrease when sepsis begins and can continue to fall until day 28.[4] Persistent lymphopenia is also associated with a poor prognosis. It was reported that persistent lymphopenia on day four after the onset of sepsis serves as a predictive factor for survival on days 28 and one year after sepsis.[5] However, thestudies assessing persistent lymphopenia in low- and middle-income countries, including in Indonesia are still limited.

Dr. Sardjito General Hospital, Yogyakarta, one of the largest university-affiliated hospitals in Indonesia, is one of the main referral hospitals in Indonesia. However, no previous study has assessed sepsis patients in relation to persistent lymphopenia. Specific hospital-based assessment would serve as important evidence to inform patient clinical management and to assess if there are any differences in the characteristics and predictive factors of mortality among sepsis patients. This study aimed to determine the clinical patterns, sociodemographics and mortality of sepsis patients with persistent lymphopenia who are treated at the Department of Internal Medicine, Dr. Sardjito General Hospital, Yogyakarta.

MATERIALS AND METHODS

Subjects

This was a descriptive study conducted at the the Department of Internal Medicine, Dr.Sardjito General Hospital, Yogyakarta. Information of patients admitted to the hospital with sepsis and persistent lymphopenia who were treated by the Department of Internal Medicine within the period of January 2016 to December 2017 were collected. The research subjects were admitted either through the emergency room or were being treated as inpatients who met the inclusion and exclusion criteria. All sociodemographic, clinical, and laboratory data including culture data from the source of infection were taken from the medical records.

The inclusion criteria were adult patients age 18 years or older who were diagnosed with sepsis and persistent lymphopenia, were treated in the emergency room or inpatient wards of the internal medicine department, and had data on lymphocyte counts on D-1 (day one) and D-4 (day four ± 24 h) following the diagnosis. The exclusion criteria included comorbidities or other factors that may affect lymphocyte levels, including HIV (human immunodeficiency virus), malignancy, autoimmune diseases, history of G-CSF (granulocyte-colony stimulating factors) consumption in the past one month, as well as history of steroid and/or immunosuppressant consumption for more than two weeks in the last one month.

Protocol of the study

Diagnosis of sepsis based on SIRS and/or qSOFA criteria. Diagnosis with SIRS criteria was a confirmed or suspected infection with at least two of four SIRS criteria. Four SIRS criteria were defined: tachycardia (heart rate > 90 beats/min), tachypnea (respiratory rate >20 breaths/min), fever or hypothermia (temperature > 38 or < 36°C), and leukocytosis or leukopenia (white blood cells >1,200/mm^3 or <4,000/mm^3). Diagnosis based on 'quick SOFA' criteria was a modified version of the Sequential (Sepsis-related) Organ Failure Assessment score. There are only three components (respiratory rate ≥ 22/min, change in mental status, systolic blood pressure ≤100 mmHg) that are each allocated one point. A qSOFA score of ≥ 2 points indicates organ dysfunction.

The lymphocyte count examination was obtained from two examinations during the treatment period, with the first examination carried out when a diagnosis of sepsis was established (lymphocyte D-1) and the second examination within 48-96 h after the first examination (lymphocyte D-4). Lymphopenia is the total lymphocyte number below the normal value that the patient has when the diagnosis of sepsis is established. The normal value based on the hematology examination tool used in Dr.Sardjito General Hospital Yogyakarta is 1.62-5.37 x 10^3/L. Persistent lymphopenia is a lymphocyteD-4 count below normal values.

Statistical analysis

The patient cohort by using descriptive statistics, including the frequency distributions, mean ± standard deviation (SD) and median was described. Kaplan-Meier survival curves using the log-rank test for patients with persistent lymphopenia was created.

RESULTS

There were 101 patients with sepsis and persistent lymphopenia who were admitted to the Dr. Sardjito General Hospital, Yogyakarta between January 2016 and December 2017 who met the inclusion and exclusion criteria. Sociodemographic characteristics are presented in TABLE 1. There were more male patients (65.3%) than female patients (34.7%). The mean age was 56.3 ± 15.51 years, where 31.7% of the sample were geriatric subjects (> 65 years). Poor nutritional status (BMI <18.5 kg/m^2) was present in 30.7% of subjects.

TABLE 1. Characteristics of sepsis patients with persistent lymphopenia

Characteristics	n (%)/mean±SD
Sex	
• Male	66 (65.3)
• Female	35 (34.7)
Age (year)	56.30± 15.51
• < 65 years	69 (68.3)
• ≥ 65 years	32 (31.7)
Nutritional Status	
• BMI < 18.5 kg/m2	31 (30.7)
• BMI ≥ 18.5 kg/m2	70 (69.3)

The mean lymphocyte D-1count was 802.10 ± 359.89/μL, and the mean D-4 lymphocyte D-4 count was 875.74 ± 356.37/μL, and the increase in the average lymphocyte counts was only 73.63/μL ± 426.86 (TABLE 2). Hypoalbuminemia occurred in 78.2% of subjects with an average albumin level of 2.76 ± 0.77 g/dL.

Only 89 (88.1%) subjects had culture data and material sensitivity from the source of infection, while the remaining 11.9% did not have culture data. From the available culture data, pathogen growth was 62%. The most common pathogens were gram negative (38.6%), followed by gram positive (32.7%) and fungi (18%), with polymicrobial infections (pathogens >1 type) occurring as much as 24.8%.

TABLE 2. Laboratory characteristics of sepsis patients with persistent lymphopenia

Characteristics	n(%)/mean±SD/ median
Lymphocytes D-1 (/μL)	802.1±359.89 [833.3(172.1 – 1560.78)]
Lymphocytes D-4 (/μL)	875.74± 356.37 [896.72(137.69 – 1600.1)]
Lymphocyte Mean Difference (/μL)	73.63±426.86
Albumin value (g/dL)	2.76 ± 0.77
Albumin Category	
• Low (<3.4 g/dL)	79 (78.2)
• Normal (≥ 3.4 g/dL)	22 (21.8)
Culture Data	
• Yes	89 (88.1)
• None	12 (11.9)
Culture positive results*	62 (61.4)
• Gram positive	33(32.7)
• Gram negative	39 (38.6)
• Fungal	18 (17.8)
• Polymicrobial (>1)	25 (24.8)
Culture negative results	39 (38.6)

*percentage>100% because there were more than one event in one subject

The clinical characteristics of the study subjects are presented in TABLE 3. The most common criteria for the diagnosis of sepsis was systemic inflammatory response syndrome (SIRS) in 70.6% of patients. This is consistent with the knowledge that new SOFA (Sequential Organ Failure Assessment) criteria were introduced in the consensus in 2015 and began to be widely used in 2018. The provision of combination antibiotics occurred in 87.1% of subjects, and only 12.9% of subjects received a single antibiotic.

TABLE 3. Clinical characteristics of sepsis patients with persistent lymphopenia

Characteristics	n (%)/ mean±SD
Diagnosis criteria	
• SIRS	72(71.3)
• SOFA	9(8.9)
• SIRS + SOFA	20(19.8)
Antibiotic combination	88(87.1)
Source of infection*	
• Respiratory tract	75(74.3
• Urinary tract	35(34.7)
• Abdominal	14(13.9)
• Skin and soft tissue	28(27.7)
• Others	8(7.9)
Multiple sources (>1)	49 (48.5)
Comorbid*	
• DM	33 (32.7)
• CKD	24 (23.8)
• Stroke	6 (5.9)
• Cirrhosis	8 (7.9)
• Heart failure	16 (15.8)
• COPD	13 (12.9)
• Hypertension	27 (26.7)
• Depression	8 (7.9)
Multiple comorbids (≥2)	33 (32.7)
Shock	30 (29.7)
Length of stay (days)	13.06 ± 6.77
Outcome	
• Alive	57 (56.4)
• Dead	44 (43.6)

* Percentage > 100% because there were more than one event in one subject

The main source of infection in this study was lung and respiratory infection (74.3%), followed by urinary tract infection (34.7%) and skin and soft tissue infections (27.7%). Multiple sources of infections were found in 48.5% of subjects. The most common comorbidities were diabetes mellitus (DM), hypertension, and chronic kidney disorders (CKD). Multiple comorbidities were found in 32.7% of the study subjects. Thirty subjects (29.7%) experienced septic shock and/or had vasopressors. The mortality rate was 43.6%. The average duration of treatment reached 13.06 ± 6.77 days. Median survival of the whole subject during treatment is presented in FIGURE 1, reaching 19 days.

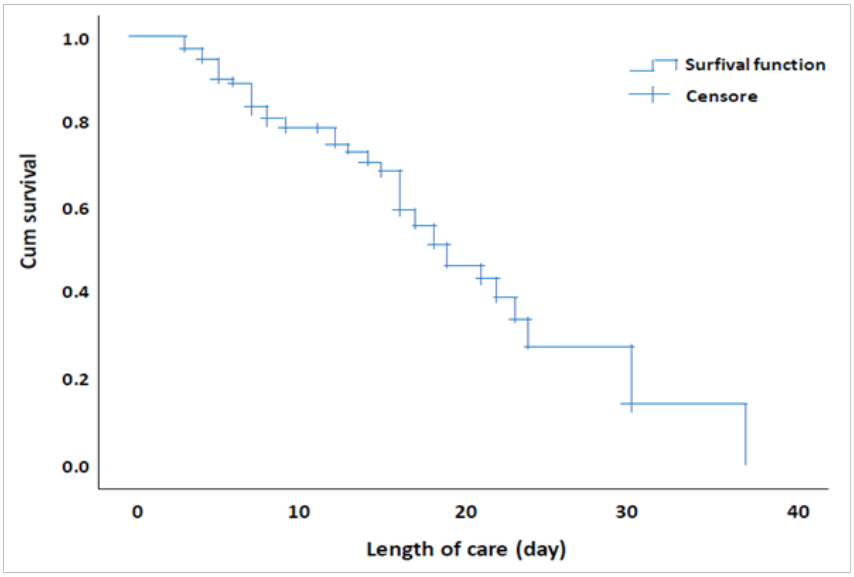

FIGURE 1. Median survival of sepsis patients withpersistent lymphopenia

DISCUSSION

In this study, male research subjects were more common than female research subjects. The mean age was 56.3 ± 15.51 years, with 31.7% of subjects being geriatric. Age and gender as risk factors for sepsis have been known from various studies. A previous study showed a higher incidence of sepsis in men at up to 1.8 times that of women, and mortality increased with increasing age over 40 years.[6] Ageing is an independent risk factor for sepsis.[7]

Additional risk factors include malnutrition, chronic diseases, immunosuppression, surgery or hospitalization and the use of catheters or other medical devices. We also observed a similar pattern in which poor nutritional status was found in 30.7% of the study subjects. Hypoalbuminemia occurred in 78.2% of subjects, and the large number of subjects with low albumin levels can be associated with the pathophysiology of hypoalbuminemia in sepsis. In sepsis, endothelial damage increases trans-capillary albumin losses, reaching 13 times from usual.[8]

The mean value of lymphocytes D-1 in the study subjects was 802.10 ± 359.89/L, and the lymphocytes D-4 were 875.74 ± 356.37/L. There are several causes of lymphopenia in addition to infection, such as malnutrition conditions, which can affect lymphocyte levels through bone marrow hypoplasia.[3] Poor nutritional status was found in 30.7% of the study subjects. Lymphopenia is also known to occur in depressed patients, although the exact mechanism of this relationship is not yet known.[9] A total of 7.9% of the study subjects had depression. Other conditions that are known to affect lymphocyte levels in the blood are heart failure, uremia, immunosuppressive conditions such as autoimmune disease, malignancy, HIV infection, and the use of drugs that affect lymphocyte production, such as corticosteroids, immunosuppressants, G-CSF, radiation, and chemotherapy.[10] In this study, the immunosuppressed conditions were part of the exclusion criteria.

The main sources of infection found

were infection of the lungs, followed by urinary tract infection and skin and soft tissue infections. This is somewhat different from other studies,[11] in which the main sources of sepsis consecutively are pulmonary, urinary and intra-abdominal infections. The most common comorbidities in our subjects were DM, hypertension, and CKD. Multiple comorbidities were found in 32.7% of the study subjects. Chronic disease patients experience chronic inflammation, which causes an increased risk of developing sepsis after exposure to infection.Previous study reported that the risk of developing sepsis in patients with comorbid chronic disease; with more comorbidities, the risk is also increasing.[12] Diabetes mellituswas the most comorbid in this study because skin and soft tissue infections became the third most common source of infection (28.5%) in this study. The most common type of skin and soft tissue infections in this study were diabetic ulcers, which were 13 of 28 subjects.

From the available culture and sensitivity data, pathogen growth was found in 61.4% of subjects. The most common pathogens were gram-negative followed by gram-positive and fungal, with polymicrobial infections (pathogens> 1 type) occurring as much as 24.8%. This is not different from other studies, where gram negativity was found as the main cause of sepsis.[8] Negative culture results were obtained from 38.6% subjects. It is in accordance with previousstudy where one-third of cultures from all sources of infection were negative.[1]However, previous study on sepsis patients at Dr.SardjitoGeneral Hospital in 2014, pathogen growth in blood cultures was only found in 30.2% of patients.[13] Negative culture results could be caused by several factors, including untimely collection of the culture materials (e.g., materials taken after empirical antibiotics have been given), prescriptions of antibiotics for the previous treatment period, anaerobic or viral or parasitic pathogens that would result in negative culture, or errors in the sampling and examination procedures.

Thirty subjects (29.7%) experienced septic shock and/or had vasopressors. The mortality rate in this study reached 43.6%, greater than recent epidemiological studies in high-income countries, where mortality in sepsis and septic shock was 17% and 25%, respectively.[6] The outcome of critically ill patients with bacteraemia sepsis was associated with age, sex, ethnicity, severity of disease at the onset of sepsis, comorbidities, source of nosocomial infection and initial cause of infection.[14]

Previous studies showedthe association between lymphopenia, sepsis and death. The presence of severe lymphopenia in septic shock patients at ICU admission associated with a 3.5-fold increase in mortality on day 28.[15] Moreover, thepersistent lymphopenia on day 4 after the onset of sepsis was a predictive factor for survival on days 28 and 1 year. As many as 22% of patients died on day28, severe persistent lymphopenia ($<0.6 \times 10^3$/L) was associated with increased secondary infections.[5]

The accumulation of pro-inflammatory cytokines is associated with death in the early phase of sepsis.[16] On the other hand, deaths due to sepsis in the later stages are related to conditions of suppression and immune dysfunction. A previous study[17]showed that most patients who survived the early phase of sepsis will enter an unresponsive immunological phase, which is characterized by a decrease in T cell function, indicating the condition of immunosuppression or immune paralysis. In fact, more than 70% of deaths due to sepsis occurred within the first 3 days, with subsequent deaths occurring within weeks of the onset of sepsis.[16] In this study, deaths occurred in 43.6% of subjects with a mean length of stay until the death outcome occurred

was 13.06 ± 6.77 days. The median survival of the whole subject during treatment was 19 days. This is consistent with deaths occurring in the later stages of sepsis related to immunosuppression and persistent lymphopenia.

This study has several limitations. First, since we relied on hospital medical records, some data were incomplete, particularly those pertaining to culture data. This may reduce the completeness of information. Second, our sample population may not necessarily represent the general population due to the single-center nature of our study setting at the hospital. Dr. SardjitoGeneral Hospital, a tertiary hospital, also has more clinical complications than patients at lower-level hospitals in Indonesia. The strength of this study is that selection bias has been minimized by exclusion criteria for immunosuppression conditions that can affect lymphocyte levels.

CONCLUSION

Septic patients with persistent lymphopenia have high mortality, and further study is needed to investigate the clinical ramifications of persistent lymphopenia.

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