Paediatrica Indonesiana

p-ISSN 0030-9311; e-ISSN 2338-476X; Vol.60, No.3(2020). p.117-24; doi: http://dx.doi.org/10.14238/pi60.3.2020.117-24

Original Article

Efficacy of high-dose methylprednisolone and cyclophosphamide in childhood-onset systemic lupus erythematosus

Putu Ayunda Trisnia, Ketut Dewi Kumara Wati, Komang Ayu Witarini, Ida Bagus Ramajaya Sutawan, Hendra Santoso

Abstract

Background Systemic lupus erythematosus (SLE) is a chronic, multisystem, autoimmune disease. Untreated SLE often become progressive and lead to increased risk of mortality. Corticosteroid and cyclophosphamide remain the treatment of choice for severe SLE. Disease activity assessed with SLE Daily Activity Index (SLEDAI).

Objective To compare the disease activity of childhood-onset severe SLE at the time of diagnosis, after completion of high dose methylprednisolone, and after three month of cyclophosphamide by using SLEDAI.

Methods This study was conducted in the Division of Pediatric Allergy and Immunology, Department of Child Health, Udayana University/Sanglah Hospital, Denpasar, Bali. Subjects were SLE patient aged 0-18 years who had severe clinical manifestations. Subject received therapy combination of high dose methylprednisolone and cyclophosphamide every 2 weeks for six doses. SLEDAI score was assessed at the time of diagnosis, after completion of high dose methylprednisolone, and after three month of cyclophosphamide.

Results During the study period, 51 children were diagnosed as SLE. Twenty-one subjects were included for analysis. Median SLEDAI score at the time of diagnosis was 23 (range 13-39). SLEDAI score after three months of cyclophosphamide was decreased to 2 (range 0-14). Post hoc analysis with Wilcoxon signed-rank test showed the improvement of SLEDAI score at the time of diagnosis and after three months of cyclophosphamide was statistically significant ($Z=-4.016$, $P<0.0001$).

Conclusion SLEDAI score reduces after completion of high-dose methylprednisolone and three month of cyclophosphamide therapy. **[Paediatr Indones. 2020;60:117-24; doi: http://dx.doi.org/10.14238/pi60.3.2020.117-24]**.

Keywords: *SLE; childhood-onset; SLEDAI; high-dose methylprednisolone; cyclophosphamide*

Systemic lupus erythematosus (SLE) is a chronic, multisystem, autoimmune disease characterized by periods of increased disease activity caused by inflammation of blood vessels and connective tissue.[1] The disease can lead to various morbidities. Clinical presentations in childhood-onset SLE are largely heterogeneous and course of disease is unpredictable. Childhood-onset SLE tend to had more severe clinical manifestation compared to adult form.[1-3] The renal and central nervous system were the organs involved and had a larger portion in the childhood-onset.[2,3] Untreated

From the Department of Child Health, Universitas Udayana Medical School/Sanglah Hospital, Denpasar, Bali, Indonesia.

Corresponding author: Ketut Dewi Kumara Wati, Department of Child Health, Udayana University Medical School, Sanglah Hospital, Pulau Nias Street, Denpasar - 80114, Indonesia. Tel. +62-361-244038; Fax +62-361-244038; Email: ketutdewi@yahoo.com.

Submitted September 17, 2019. Accepted April 28, 2020.

SLE becomes progressive and increase risk of mortality. Approximately twenty percent of SLE cases develop during the first two decades of life.1,2,4 The prevalence of childhood-onset SLE was 6.3-19.3 per 100,000 in Asia. The mean age at diagnosis was 8.6–13.5 years.[4] From 2005 through 2013, 91 children with SLE were hospitalized in Dr. Cipto Mangunkusumo Hospital, Jakarta.[5]

Corticosteroids and cyclophosphamide remain the treatment of choice for severe SLE (SLE with significant renal/central nervous system/cardiac involvement).[6] The use of corticosteroids and immunosuppressive therapy was recommended by *European League Against Rheumatism* (EULAR) guidelines.[6,7] Long-term monitoring was recommended to evaluate the disease activity and adverse effect therapy, with 3-6 month assessment for mild disease and more frequent in patients with severe active disease.[7-10]

Disease activity cannot be determined by a single clinical sign or laboratory. Different measures have been developed to assess SLE disease activity including the systemic lupus activity measure (SLAM), SLE disease activity index (SLEDAI), the European consensus lupus activity measurement (ECLAM), and the British isles lupus assessment group (BILAG).[11] Overall, these disease activity measurements are accurate and reliable. SLEDAI score system easy to use, even for beginner. SLEDAI can be completed in about two minutes. Every variable is clearly defined to minimize misperception. SLEDAI score also sensitive to assess changes in disease activity. The final SLEDAI score would be from 0 (no disease activity) and 105 (the most severe disease activity). An increased in SLEDAI > 3 were considered as increased disease activity.[12]

This study aimed to compare the disease activity of childhood-onset severe SLE at the time of diagnosis, after completion of high dose methylprednisolone, and after 3 months of cyclophosphamide by using SLEDAI. Our hypothesis is there was an improvement of SLEDAI after high dose methylprednisolone and after three months of cyclophosphamide.

Methods

This study was conducted in the Division of Pediatric Allergy and Immunology, Department of Child Health, Medical School, Udayana University/Sanglah Hospital Denpasar, Bali. Data were obtained from patients medical records. We included children with severe SLE (SLE with severe clinical manifestations) based on *American College of Rheumatology/Systemic Lupus International Collaborating Clinics* (ACR/SLICC) *2012 Criteria*,[13] aged 0-18 years, from July 2015 to December 2018. Exclusion criteria were incomplete medical records and subjects who did not finish induction therapy according to protocol for severe SLE (high-dose methylprednisolone and 6 intravenous cyclophosphamide in 2-week interval).

Consecutive random sampling was performed until the minimum subject fulfilled. The minimum required number of subjects was calculated with standard deviation 2.2,[14] type I error 5%, power 80%, and mean difference 2. Data collected from the patients' medical records included age, date of diagnosis, sex, nutritional status, antinuclear antibody immunofluorescence (ANA IF), anti-dsDNA antibody, complement 3 (C3), and disease activity. Disease activity was assessed based on SLEDAI score. SLEDAI was calculated at the time of diagnosis, after high-dose methylprednisolone and after completion of intravenous cyclophosphamide. Age was defined as the chronological age stated in years. For those aged > 6 months, age was rounded up; for those aged ≤ 6 months, age was rounded down. Nutritional status was determined based on weight-for-height. Nutritional status was classified according to the *Center for Disease Control and Prevention* (CDC) 2000[15] and Waterlow criteria (weight/ideal body weight)15 as: (1) obesity > 120%, (2) overweight 111-120%, (3) well-nourish 90-110%, (4) moderate malnutrition 70-89%, (5) severe malnutrition < 70%. Short stature was defined as height-for-age below -2 standard deviation. The data of ANA IF, anti-dsDNA antibody, and C3 was obtained from subjects' medical records based on Prodia laboratory examination. ANA IF was negative if the titer ≤ 1:100 and positive if the titer > 1:100. Anti-dsDNA was determined quantitatively. Complement 3 was not checked routinely in all SLE patients due to financial issues.

The SLEDAI score was evaluated using *SLEDAI 2000/SLEDAI 2K Sheet*, which consists of 24 parameters (clinical and laboratory). The minimum SLEDAI score is 0, the maximum score is 105. An

increase in SLEDAI > 3 were considered as increased disease activity.[12] Severe SLE defined as SLE with organ involvement that may lead to irreversible damage in the affected organ, such as lung, cardiac, renal, central nervous system (CNS), and hematology involvement.[6,16] Therapy was started once severe SLE was diagnosed. Induction therapy consists of intravenous methylprednisolone 30 mg/kg/day (maximum 1000 mg) for three consecutive days, followed by cyclophosphamide 500 mg every 2 weeks for six doses (3 months).

Statistical analysis was performed using *SPSS version 20.0* (*SPSS INC*., Chicago). Mean SLEDAI at the time of diagnosis, after high dose methylprednisolone, and after three month of cyclophosphamide were compared by using Friedman test. A P value of <0.05 was considered statistically significant. This analysis was continued by Wilcoxon post-hoc test. The study protocol had been approved by the Research Ethics Commission of Universityas Udayana Medical School, Denpasar, Bali.

Results

During the period of July 2015 until December 2018, there were 51 SLE patients, 39 patients were diagnosed as severe SLE, but only 21 patients were included in the study. Eighteen patients were excluded (ten patients died before completing the induction therapy, five patients continued therapy in another

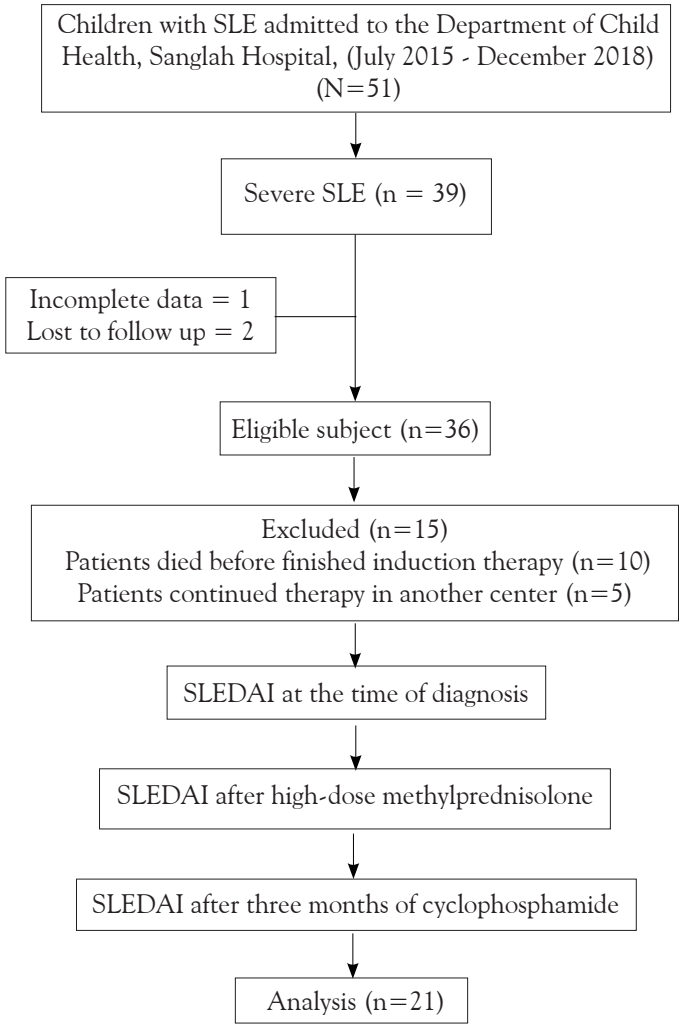

Figure 1. Study flow chart

center, one patient with incomplete medical record, two patients lost to follow up). The study flow chart is shown in **Figure 1**.

Characteristics of subject is shown in **Table 1**. The mean age at diagnosis was twelve year, the youngest subject was eight years old and the oldest subject was seventeen years old. Eighteen (85.7%) subjects were female and three (14.2%) subjects were male, the ratio of female and male was 18:3. Eight (38%) patients were malnourish whereas nine (42.8%) patients were short stature.

Table 1. Subjects characteristic

Clinical characteristics	(N=21)
Mean age at diagnosis (SD), years)	12.47 (2.47)
Age group, n	
5-10 year	1
10-18 year	20
Sex, n	
Male	3
Female	18
Nutritional status, n	
Obesity	1
Overweight	3
Well-nourish	9
Moderate malnutrition	8
Height per age, n	
Normal	12
Short stature	9
ANA IF, n	
Positive	21
Median anti-dsDNA (range), IU/mL	790 (10 – 3200)

The most clinical manifestations observed according to SLICC criteria was renal manifestation (85.7%), followed by non-scarring alopecia (76.1%), serositis (71.4%), and acute cutaneous lupus (61.9%). Neurological manifestation was found in 23% of subjects. **Table 2** shows organ involvement during clinical course of severe SLE.

Positive ANA test was found in all subject. An increased of anti-dsDNA were found in eighteen subject (85.7%). Hemolytic anemia observed in four cases (19%). Leukopenia and thrombocytopenia were found in only two subjects. Smith antibodies (anti-Sm) were found in four subjects. From twenty-one subjects, seventeen subjects underwent C3 examination. The mean C3 level at the time of diagnosis was 37.4 mg/dL.

Table 2. Major organ involvement

Organ	n
Central nervous system	5
Cardiovascular	5
Lung	5
Renal	17

According to **Table 3**, the median SLEDAI score at the time of diagnosis was 23 (13-39) and decreased to 2 (0-14) after three months of cyclophosphamide. There was a significant difference in SLEDAI score ($P<0.0001$) between subjects' group (**Table 4**).

Table 3. SLEDAI over three periods of observation

SLEDAI score	Median (range)
Total SLEDAI score at the time of diagnosis	23 (13-39)
Total SLEDAI score after high-dose methylprednisolone	13 (0-21)
Total SLEDAI score after three month of cyclophosphamide	2 (0-14)

Table 4. Analysis of total SLEDAI of each group

SLEDAI	Mean rank	P value*
Total SLEDAI at the time of diagnosis	3.00	<0.0001
Total SLEDAI after high-dose methylprednisolone	1.98	
Total SLEDAI after three month of cyclophosphamide	1.02	

*Friedman test

To determine when differences actually occured in the clinical course, we run the post-hoc Wilcoxon analysis on the different combinations of the related groups (**Table 5**). There were statistically significant improvement of total SLEDAI score between the time of diagnosis and after high-dose methylprednisolone, the time of diagnosis and after three months of cyclophosphamide, and between after high-dose

Table 5. Relationship between observed groups

SLEDAI	Score change(Z)	P value*
Total SLEDAI at the time of diagnosis – after high-dose methylprednisolone	-4.018**	<0.0001
Total SLEDAI at the time of diagnosis – after 3 months of cyclophosphamide	-4.016**	<0.0001
Total SLEDAI after high-dose methylprednisolone - after 3 months of cyclophosphamide	-3.923**	<0.0001

*Wilcoxon signed rank test **Based on positive ranks

methylprednisolone and after three months of cyclophosphamide.

Discussion

The mean age of SLE onset in this study was twelve years, with most subjects diagnosed above ten years of age. Several studies found a similar result. A previous stud reported the mean age of disease onset was 10.5 years, with the most prevalent age > 10 years,[17] while another study reported the mean age at diagnosis was 11.9 years.[2] The two studies mentioned above reported that female: male ratio was 4.5:1 and 5:1, respectively.[2,17]

The most clinical manifestations observed according to SLICC criteria were renal manifestation (85.7%), followed by non-scarring alopecia (76.1%), serositis (71.4%), and acute cutaneous lupus (61.9%). Neurological manifestation was found in 23% of subjects. A previous study reported the most common clinical finding was hematologic manifestations, followed by arthritis and malar rash.[2] Another study reported that the most common symptoms were rash on skin/face and arthralgia.[17] In this study, 61.9% of subjects were having acute cutaneous lupus. Mucocutaneous manifestation was reported up to 90% of pediatric SLE. Malar or "butterfly" rash is pathognomonic for SLE.[1] The common organ involved in childhood-onset SLE was the kidney. A study reported 82% of childhood-onset SLE among Vietnamese had renal manifestation.[18] Another study reported 62.8% of their subject had renal manifestation.[19] In contrast, a study in Singapore reported the renal involvement was only 40%.[2] Therefore periodic renal evaluation should be performed in all childhood-onset SLE.

Positive ANA test was found in all subjects. A previous study reported nearly all (98%) their subjects had positive test.[2] Another studies reported positive ANA test in about 91% of childhood-onset SLE had positive ANA.[17,20] Positive ANA test has a high sensitivity of 98%, but low positive predictive value (10%) and specificity as low as 36%.[21] Several disease or syndrome frequently associated with a positive ANA test are SLE, juvenile rheumatoid arthritis, juvenile dermatomyositis, mixed connective tissue disease, psoriatic arthritis, infection (Lyme), and malignancy (acute lymphocytic leukemia).[1,17,20-23] ANA was comprised as collections of autoantibodies against various nuclear proteins. The presence of high titers of ANA should arouse suspicion of autoimmune disease.[23] Positive ANA titers were categorized as low (1:40 to 1:80), medium (1:160 to 1:320), and high (≥ 1:640).[23] Up to 10% of healthy children and adolescent may present with isolated high titer of ANA.[23] Low ANA titer (< 1:640) should be ignored unless the child is systemically ill and show clinical manifestations of SLE.[21] A study found that children with high ANA titers have a higher mortality risk.[23]

In this study, anti-dsDNA were found in eighteen subject (85.7%), anti-Sm (19%), and anti-ribosomal P (9.5%). A study found 85% of childhood-onset SLE had positive anti-dsDNA.20 Anti-dsDNA, anti-Smith antibodies (anti-Sm), and anti-ribosomal P autoantibodies are pathognomonic of SLE diagnosis. Other autoantibodies that may be found at SLE are anti-RNP, anti-SSA/Ro, and anti-SSB/La.[24,25] Anti-ds DNA, found in > 75% of childhood-onset SLE and can be used as a parameter of SLE activity.1,20 Anti-dsDNA antibodies in SLE has high specificity. Anti-Sm has the highest specificity, but low sensitivity for SLE. Both anti-dsDNA and anti-Sm antibodies are associated with renal involvement.[26] In this study, from eighteen subjects who have positive anti-ds DNA, only one subject did not have renal manifestation. In contrast, a previous study found that anti-dsDNA was not associated with increased frequency of renal involvement. Two subjects have strong positive anti-ribosomal P. The organ involved in both subjects was central nervous system, with seizure as the chief complaint. One subject has strong positive anti-SSA, anti-SSB, and recombinant Ro52. The main clinical manifestation in this subject was large pericardial effusions and anemia. One subject has strong positive anti-RNP/SM and anti-Sm, where the symptom is predominantly in the central nervous system and liver.[2]

Reduced serum complements C3 and C4 have been used as a marker of active lupus disease relapse for decades.[1,25] A previous study found the median C3 and C4 level were 56 (range 9-173) mg/dL and 8 (range 2-35)mg/dL, respectively. In this study, the median C3 level when SLE confirmed was 37.4 (range 17-85) mg/dL.[2]

The aim of SLE management was to achieve

remission, reduces disease activity, and prevent toxicities from medications. The approach of management of childhood-onset SLE was achieved by using glucocorticoids and immunosuppressive drugs. In our center, severe SLE is treated with intravenous methylprednisolone 30 mg/kg to a maximum of 1 gram (for three consecutive days) then followed with glucocorticoids 2 mg/kg/day. Cyclophosphamide is primarily used as immunosuppressive drugs in severe SLE, including lupus nephritis, life-threatening organ involvement, and neuropsychiatric manifestations. There are two regimens of intravenous cyclophosphamide recommended for induction therapy in lupus nephritis. The first one is low dose cyclophosphamide (500 mg intravenous once every 2 weeks for a total of 6 doses), followed by maintenance therapy with daily oral azathioprine or daily oral mycophenolate mofetil. The second one is high dose cyclophosphamide (500-1000 mg/m^2 intravenous once a month for 6 doses), followed by mycophenolate mofetil or azathioprine.[27] The European protocol for management of lupus nephritis consist of low-dose intravenous cyclophosphamide (500 mg) every 2 weeks for six doses. This regimen was effective for the initial treatment.[28]

We evaluate SLEDAI score at the time of diagnosis, after patients had three consecutive high-dose intravenous methylprednisolone, and after six doses of low-dose intravenous cyclophosphamide. Median SLEDAI at the time of diagnosis was 23 (range 13-39), after 3 consecutive high-doses of methylprednisolone was 13 (range 0-21), and after 3 months of cyclophosphamide was 2 (range 0-14). A previous study found the mean SLEDAI at the time of diagnosis was 13.[29] The subjects with both renal and CNS manifestations show an improvement after 6 months therapy, where the mean SLEDAI decrease from 18.2 (SD 10.5) to 1.9 (SD 1.7) (P=0.0001).[29] Another study also found an improvement of SLEDAI 6 months after induction therapy where the decrease of SLEDAI was 11.07.[11] A study observed SLEDAI at the time of diagnosis and then repeated every 3 months for about 12 months of observation. They found the median SLEDAI at time of diagnosis was 16 (range 8-34), and it decreased 3 months later.[5]

The Friedman test found the difference of SLEDAI score among all three groups was statistically significant (P<0.0001). Post hoc analysis with Wilcoxon signed-rank test found there was an improvement of SLEDAI score at the time of diagnosis and after high-dose methylprednisolone Z=-4.018; P<0.0001), at the time of diagnosis and after 3 months of cyclophosphamide (Z=-4.016; P<0.0001), after high-dose of methylprednisolone and after 3 months of cyclophosphamide (Z=-3.923; P<0.0001). These analysis shows that SLEDAI after high-dose methylprednisolone and after three month of cyclophosphamide was improved significantly.

There were several limitation in this study. Some laboratory parameters need to assess according to SLEDAI such as DNA binding and complement level were not checked routinely due to clinical judgement and cost. Also, the small sample size limited the strength of the study. In conclusiom, SLEDAI scores reduce after completion of high-dose methylprednisolone and three month of cyclophosphamide therapy.

Conflict of interest

None declared

Acknowledgements

We thank our colleagues from Department of Child Health, Universitas Udayana Medical School/ Sanglah Hospital who provided insight and expertise that greatly assisted the study.

Funding acknowledgements

The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Weiss JE. Pediatric systemic lupus erythematosus: more than a positive antinuclear antibody. Pediatrics in Review. 2012;33:62-74. DOI: 10.1542/pir.33-2-62.
2. Tan JHT, Hoh SF, Win MTM, Chan YH, Das L, Arkachaisri T. Childhood-onset systemic lupus erythematosus in Singapore: clinical phenotypes, disease activity, damage, and autoantibody profiles. Lupus. 2015;0:1-8.

DOI: 10.1177/0961203315584413.

3. Fatemi A, Matinfar M, Saber M, Smiley A. The association between initial manifestations of childhood-onset systemic lupus erythematosus and the survival. International Journal of Rheumatic Diseases. 2015;1-7. DOI: 10.1111/1756-185X.12807.
4. Huang JL, Yeh KW, Yao TC, Huang YL, Chung HT, Ou LS, *et al.* Pediatric lupus in Asia. Lupus. 2010;19:1414-8. DOI: 10.1177/0961203310374339.
5. Saleh AM, Kurniati N, Syarif BH. Penilaian aktivitas penyakit lupus eritematosus sistemik dengan skor SLEDAI di Departemen Ilmu Kesehtan Anak RSCM. Sari Pediatri. 2014;16:292-8. DOI: 10.14238/sp16.4.2014.292-8.
6. Fanouriakis A, Kostopoulou M, Alunno A, Aringer M, Bajema I, Boletis JN, *et al.* 2019 Update of the EULAR recommendations for the management of systemic lupus erythematosus. Ann Rheum Dis. 2019;78:736-45. DOI: 10.1136/annrheumdis-2019-215089.
7. Tunnicliffe DJ, Singh-Grewal D, Kim S, Craig JC, Tong A. Diagnosis, monitoring, and treatment of systemic lupus erythematosus: a systematic review of clinical practice guidelines. Arthritis Care & Research. 2015;67:1440-52. DOI: 10.1002/acr.22591.
8. Nazri SKSM, Wong KK, Hamid WZWA. Retrospective analysis of clinic-laboratory parameters and their association with systemic lupus erythematosus disease activity index score. Saudi Med J. 2018;39:627-31. DOI: 10.15537/smj.2018.6.22112.
9. Zhang XW, Li C, Ma CC, Zhao JX, An Y, Liu S, *et al.* Short-interval lower-dose intravenous cyclophosphamide as induction and maintenance therapy for lupus nephritis: a prospective observational study. Clin Rheumatol. 2014;33:939-45. DOI: 10.1007/s10067-014-2590-6.
10. Houssiau FA, Vasconcelos C, D'Cruz D, Sebastiani GD, Garrido ER, Danieli MG, *et al.* Early response to immunosuppressive therapy predicts good renal outcome in lupus nephritis. Arthritis & Rheumatism. 2004;50:3934-40. DOI: 10.1002/art.20666.
11. Brunner HI, Feldman BM, Bombardier C, Silverman ED. Sensitivity of the Systemic Lupus Erythematosus Disease Activity Index, British Isles Lupus Assessment Group Index, and Systemic Lupus Activity Measure in the evaluation of clinical change in childhood-onset systemic lupus erythematosus. Arthritis & Rheumatism 1999;42:1354-60. DOI: 10.1002/1529-0131(199907)42:7<1354::AID-ANR8>3.0.CO;2-4.
12. Griffiths B, Mosca M, Gordon C. Assessment of patients with systemic lupus erythematosus and the use of lupus disease activity indices. Best Practice & Research Clinical Rheumatology 2005;19:685-708. DOI: 10.1016/j.berh.2005.03.010.
13. Salehi-Abari I. 2015 ACR/SLICC revised criteria for diagnosis of systemic lupus erythematosus. Autoimmune Dis Ther Approaches. Open Access 2015;2:1-4. DOI: 10.14437/ADTAOA-2-114.
14. Pusongchai T, Jungthirapanich J, Khositseth S. Pediatric systemic lupus erythematosus in Thammasat University Hospital. J Med Assoc Thai. 2010;93(Suppl.7):S283-93. PMID: 21294427.
15. Ikatan Dokter Anak Indonesia (IDAI). Rekomendasi ikatan dokter anak Indonesia: asuhan nutrisi pediatrik (pediatric nutrition care). Jakarta: Ikatan Dokter Anak Indonesia; 2011.
16. Rachmawati P, Murni IK, Nugroho S, Noormanto, Sumadiono. Factor associated with pericardial effusion in pediatric systemic lupus erythematosus. Pediatr Indones. 2018;58(5):227-32. DOI: 10.14238/pi58.5.2018.227-32.
17. Munasir Z, Mariana T, Suradi R. Clinical and laboratory features of childhood systemic lupus erythematosus and its course in Department of Child Health, Cipto Mangunkusumo Hospital, Jakarta. Pediatr Indones. 2001;41:214-24. DOI: 10.14238/pi41.4.2001.214-24.
18. Dung NTN, Loan HT, Nielsen S, Zak M, Petersen FK. Juvenile systemic lupus erythematosus onset patterns in Vietnamese children: a descriptive study of 45 children. Pediatric Rheumatology. 2012;10:38. DOI: 10.1186/1546-0096-10-38.
19. Gulay CB, Dans LF. Clinical presentations and outcomes of Filipino juvenile systemic lupus erythematosus. Pediatric Rheumatology. 2011;9:7. DOI: 10.1186/1546-0096-9-7.
20. Faisal S, Akib A, Tambunan T. Clinical and laboratory manifestations of childhood and adult-onset systemic lupus erythematosus in Cipto Mangunkusumo Hospital. Paediatr Indones. 2003;43:199-204. DOI: 10.14238/pi43.6.2003.199-204.
21. Malleson PN, Mackinnon MJ, Sailer-Hoeck M, Spencer CH. Review for the generalist: the antinuclear antibody test in children – when to use it and what to do with the positive titer. Pediatric Rheumatology. 2010;8:27. DOI: 10.1186/1546-0096-8-27.
22. Egner W. The use of laboratory tests in the diagnosis of SLE. J Clin Pathol. 2000;53:424-32. DOI: 10.1136/jcp.53.6.424.
23. Chou IJ, Kuo CF, See LC, Hsia SH, Yu KH, Luo SF, *et al.* Antinuclear antibody status and risk of death in children and adolescents. Scand J Rheumatol. 2011;40:472-7. DOI: 10.3109/03009742.2011.593546.
24. Silva CA. Childhood-onset systemic lupus erythematosus: early disease manifestations that the paediatrician must know.

Expert Review of Clinical Immunology. 2016;12:907-10. DOI: 10.1080/1744666X.2016.1195685.

25. Binder E, Edelbauer M. Use of biomarkers in the management of children with lupus. Curr Rheumatol Rep. 2013;15:312. DOI: 10.1007/s11926-012-0312-0.
26. Levy DM, Kamphuis S. Systemic lupus erythematosus in children and adolescents. Pediatr Clin North Am. 2012;59:345-64. DOI: 10.1016/j.pcl.2012.03.007.
27. Hahn BH, McMahon M, Wilkinson A, Wallace WD, Daikh DI, Fitzgerald J, *et al.* American College of Rheumatology Guidelines for screening, treatment and management of lupus nephritis. Arthritis Care Res (Hoboken). 2012;64:797-808. DOI: 10.1002/acr.21664.
28. Thakral A, Klein-Gitelman MS. An update on treatment and management of pediatric systemic lupus erythematosus. Rheumatol Ther. 2016;3:209-19. DOI: 10.1007/s40744-016-0044-0.
29. Hiraki LT, Benseler SM, Tyrrell PN, Hebert D, Harvey E, Silverman ED. Clinical and laboratory characteristics and long-term outcome of pediatric systemic lupus erythematosus: a longitudinal study. J Pediatr. 2008;152:550-6. DOI: 10.1016/j.jpeds.2007.09.019.