

## Pattern of Indirect Immunofluorescence Assay Antinuclear Antibody in Pediatric Lupus Nephritis

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### Abstract

**Objective:** To determine the association between Anti-Nuclear Antibody (ANA) pattern in pediatric Systemic Lupus Erythematosus (SLE) patients and proteinuria as a sign of renal involvement in SLE.

**Methods:** This was a cross-sectional study, using data from medical records involving 89 newly diagnosed with SLE (aged  $\leq 18$  years) in Department of Child Health Dr. Hasan Sadikin General Hospital, Bandung, from January 1, 2018 to June 30, 2019. Data of ANA pattern and proteinuria were collected from medical record. ANA pattern was examined by Immunofluorescence Assay (IFA) method. Chi-square was used to analyze the association between ANA pattern and proteinuria as a sign of renal involvement in pediatric SLE patients.

**Results:** There were 89 patients, consisting of 7 male (7.9%) and 82 female (92.%) with median age of 14 (IQR=12–16). There were only 44 pattern of ANA as follow: homogenous 56.8%, speckled 36.4%, and nucleolar 6.8%. However, there is no significant association between homogenous pattern with proteinuria events ( $p=0.831$ ).

**Conclusions:** Homogenous pattern was the most frequent ANA pattern in children with SLE, and the pattern has no association with proteinuria events.

**Keywords:** Antinuclear antibody pattern, indirect immunofluorescence assay; proteinuria, systemic lupus erythematosus

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### Introduction

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease characterized by a loss of tolerance from the immune system and formed autoantibodies. The autoantibody also known as Anti-Nuclear Antibody (ANA) which have many subtypes that can attack self-antigens and cause inflammation in several organs, such as skin, joints, kidneys, blood-forming cells, blood vessels, and central nervous system.<sup>1,2,3,4</sup> ANA test is one of tests that used for helping diagnose autoimmune

disorder. Patients with SLE are almost always positive for ANA, meanwhile patients with other autoimmune disorders who have positive ANA result are varies, even a significant number patients with other types of disorders and some healthy people might have positive ANA, especially at low levels. Moreover, American College of Rheumatology (ACR) 1997 recommends Immunofluorescence Assay (IFA) method as a gold standard of ANA test which can help clinician to diagnose the autoimmune disorder more specifically besides another manifestations showed by the patient. It can also help to reduce risk of false positive or erroneous.<sup>1</sup> By the result of the test, the clinician might see the titers and different types of ANA through the staining pattern. Based on International Consensus of ANA Pattern (ICAP), ANA is divided into

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three major categories, namely nuclear (such as homogeneous, speckled, centromere, nucleardots and nucleolar), cytoplasmic and mitotic patterns. The results of the titer and ANA pattern will be varied in each patients.<sup>7,15,16</sup>

Systemic Lupus Erythematosus can occur in both adults and children. However, when compared with adults, children and adolescents have a higher risk of severity and involvement of other organs as a manifestation in SLE.<sup>5,6</sup> The most common and difficult manifestation of SLE is renal involvement. The involvement of the renal as a clinical manifestation will cause inflammation in the renal called nephritis.<sup>5,6,8</sup> One of the result of the inflammation is the presence of protein in the urine, which called proteinuria. In the case of pediatric SLE, lupus nephritis is more common in females than males with a ratio of 8-13: 1 and in the age range of 14-16 years.<sup>5</sup> Also, pediatric SLE with nephritis having poorer prognosis than others without nephritis.<sup>8,9</sup>

The study about relevance of ANA in SLE has been addressed repeatedly. Through the studies, ANA is known to have a correlation with the clinical manifestations of SLE, especially in patient with positive anti-

dsDNA and/or anti-Sm. The presence of anti-dsDNA was found to be associated with renal disorder, haematological involvement and serositis, meanwhile patient with anti-Sm had the highest frequency of malar rash, oral ulcers, arthritis, and serositis.<sup>18</sup> Whereas, studies about ANA pattern and its relation with the clinical manifestation of SLE are very scarce especially proteinuria. A few studies stated, there were no association between ANA Pattern and clinical manifestation of SLE, Frodlund M et al. observed that some pattern may have an association with the clinical manifestation of SLE.<sup>10,12,13,14</sup> However, this finding needs more confirmation by others.<sup>10,14,17,18</sup>

This study is aimed to determine the association between ANA pattern in pediatric SLE patients and proteinuria as a sign of renal involvement in Department of Child Health Hasan Sadikin Bandung General Hospital.

## Methods

This was a cross-sectional study conducted of 89 subject newly diagnosed patients with Systemic Lupus Erythematosus (aged ≤18 years), in Department of Child Health Dr. Hasan Sadikin General Hospital, Bandung, from January 1<sup>st</sup> 2018 until June 30<sup>th</sup> 2019. Secondary data from medical records of newly diagnosed with SLE were used in this study. Data of age, gender, ANA pattern, and proteinuria were collected from the medical records. ANA pattern was examined by Immunofluorescence Assay Method. Data was taken after obtaining Ethical Clearance issued by Health Research Ethics Committee of Universitas Padjadjaran Bandung No. 719/UN6.KEP/EC/2019 and Research Licensing Letter issued by Research Ethics Committee of Dr. Hasan Sadikin General Hospital Bandung No. LB.02.01/X.2.2.1/10047/2019. Microsoft® Excel 2013 and IBM® SPSS® Version 20 were used to process the data. Chi-square was used to analyze the association between ANA pattern and proteinuria as a sign of renal involvement in SLE and p<0.05 was considered statistically significant.

## Results

There were 89 subjects, consisting of 7 male and 82 female with median age of 14 (IQR=12–16) (see Table 1). Besides hematological manifestation, renal was the most frequent manifestation in pediatric SLE patients (see Table 1). Pattern of ANA was analyzed and

**Table 1 Characteristics of Systemic Lupus Erythematosus Patients**

Characteristics	Total (n=89)
Age (years)	
Median [IQR]	14 (12-16)
Gender (n, %)	
Boys	7 (7.9%)
Girls	82 (92.1%)
Skin manifestation (n, %)	
Malar Rash	32 (44.4%)
Discoid Rash	14 (22.6%)
Photosensitivity	29 (36.7%)
Mouth Ulcers	12 (19.7%)
Renal manifestation (n, %)	
Proteinuria	46 (51.7%)
Hematuria	42 (47.2%)
Hematological manifestation (n, %)	
Anemia	61 (77.2%)
Leukopenia	19 (24.1%)
Thrombocytopenia	10 (12.7%)
Neuropsychiatric manifestation (n, %)	7 (9.2%)

**Table 2 ANA Pattern of Systemic Lupus Erythematosus Patients**

Category	Total (n=44)
Homogenous (n, %)	25 (56.8%)
Speckled (n, %)	16 (36.4%)
Nucleolar (n, %)	3 (6.8%)

homogenous was the most common type followed by speckled, and nucleolar (Table 2). Further data processing was performed with chi-square to assess the association between ANA pattern and proteinuria events as a sign of renal involvement in pediatric SLE patients (Table 3). There is no significant association between homogenous pattern with proteinuria events.

### Discussion

Based on the study, it is known that the subjects are mostly girls. Similar with the result of other studies that have been done, one of them is study from Almaani S, et al. which stated that the most of children with SLE were girls than boys, with female-to-male ratio range between 8-13 : 1 and in the age range of 14-16 years.<sup>5</sup> This finding was because of hormonal factors such as sex steroids which are owned by girls, thus will increasing the risk of SLE.<sup>1,6</sup>

In patients with SLE, we can find the presence of ANA. ANA is autoantibodies which formed by the immune system that can attack self-antigen and cause inflammation in many tissues and organs.<sup>1,2,3,4,5</sup> ANA test is a primary test to determines the presence of ANA. Despite this, ACR 1997 recommends Immunofluorescence Assay (IFA) method as a gold standard of ANA test, because it can help the clinician to diagnose the patient more specifically and reduce risk of false positive or erroneous based on the results of the test. From the test, the clinician might see the titers and patterns ANA of the patient.<sup>7,15,16,19</sup> However, only few patients are checked for

ANA with IFA method, while the rest patients only reported whether ANA is reactive or non-reactive.<sup>10,11</sup>

According to the ICAP, ANA have associated with some autoimmune diseases which show based on the staining pattern. Homogeneous patterns was common in SLE, drug-induced lupus, and Juvenile Idiopathic Arthritis (JIA) patients, while speckled was common in Mixed Connective Tissue Disease (MCTD), SLE, Sjogren Syndrome (SJS), and Systemic Sclerosis (SSc) patients.<sup>15,17</sup> In this study, ANA pattern of the patients only homogeneous, speckled, and nucleolar. The most frequent pattern was homogeneous (56.8%) followed by speckled (36.4%), and nucleolar (6.8%). This is similar with the study of Yilmaz O et al. which reported homogenous pattern were the most common patterns, the result of the study showed that homogenous pattern in more than half of their patients.<sup>13</sup> But differ with the study of Ghrahani R, et al. which are speckled (40.3%) was the most frequent of ANA pattern, followed by nucleolar (16.1%), nucleardot (9.7%), and combination of nucleardot with speckled (3.2%).<sup>10</sup> Also, Mengeloglu Z, et al. reported speckled patterns were the most common patterns.<sup>12</sup>

Frodlund M et al. observed that ANA is known to have a correlation with the clinical manifestations of SLE, especially in patient with positive anti-dsDNA and/or anti-Sm. The presence of anti-dsDNA was found to be associated with renal disorder, haematological involvement, and serositis, meanwhile patient with anti-Sm had the highest frequency of malar rash, oral ulcers, arthritis, and serositis. Some ANA pattern may have an association with the clinical manifestation of SLE. Organ damage was less common among patients with speckled pattern. However, this finding needs more confirmation by others.<sup>18</sup>

There is no association between ANA pattern with the proteinuria events in this study. This result is similar with the study of Ghrahani R, et al. which stated that there was no association between ANA pattern

**Table 3 Association between ANA Pattern and Proteinuria Events**

Category	Proteinuria		P	OR (95% CI)
	Positive	Negative		
ANA Pattern (n=44)				
Homogenous	15	12	0.831*	0.875 (0.256-2.989)
Non - homogenous	10	7		

\*chi-square test, OR: Odds Ratio, CI: Confidence Interval

with the proteinuria events with chi-square test  $p=0.680$ .<sup>10</sup> It can be said that there is no difference with the studies even though being conducted in the different periods and the same place. Damoiseaux J, et al stated that there are several reasons for not finding an association between the ANA pattern and the manifestations of the disease. First, determination of the patterns in laboratories is inconsistent because of the subjectivity and experienced of the staff in interpretation the results. Second, healthy individual may have autoantibodies. Third, the result of the patterns may slightly differ depending on the substrate used.<sup>14,17</sup>

This study had several limitations, the

data was collected retrospectively and not all samples have the variables needed (i.e. Anti Nuclear Antibody Pattern),

Based on this study, we concluded that ANA can be checked by IFA. By the test, clinician might see the different types of ANA through staining pattern. In this study, homogenous pattern was the most frequent ANA pattern in patient with SLE. Renal involvement was common in children and adolescents compared to adults patient with SLE, besides hematological involvement. Involvement of the renal can cause inflammation and one of the result is the presence of proteinuria. The homogeneous pattern has no association with proteinuria events.

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