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Diagnostic Value of Urinary Dysmorphic Erythrocytes in SLE Patients with Three Different Methods

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ABSTRACT

Systemic Lupus Erythematosus (SLE) is an autoimmune disease with various clinical manifestations. Lupus nephritis is the most common severe manifestation with a poor prognosis. Hematuria is included in the Lupus Activity Criteria Count (LACC) and SLE Disease Activity Index (SLEDAI). Phase Contrast Microscope (PCM) availability as a recommended instrument for dysmorphic erythrocytes evaluation is exclusive, thus causing this examination to be performed rarely. This study aimed to investigate the diagnostic value of dysmorphic erythrocytes in SLE patients with hematuria using Low Condenser Light Microscope (LCLM), PCM, and UF-500i. This research was a cross-sectional study with consecutive sampling; 58 fresh urine samples were examined with UF-500i during May-July 2019. Percentage of dysmorphic erythrocytes were evaluated using LCLM and PCM. Difference percentages of dysmorphic erythrocytes were analyzed using the Wilcoxon Signed Ranks test, degree of agreement by Kappa coefficient, cut-off, sensitivity, and specificity by ROC curve. Dysmorphic erythrocyte percentage in LCLM and PCM showed a significant difference ($p < 0.001$) and a low degree of agreement (Kappa=0.373). Dysmorphic erythrocyte cut-off with LCLM was 7.5% (sensitivity 70%, specificity 68%) and PCM was 6.5% (sensitivity 74%, specificity 65%). Dysmorphic? flagging from UF-500i showed a sensitivity, specificity, PPV, NPV of 78%, 52%, 58% and 73%, respectively. LCLM can be considered a substitute for PCM for evaluating dysmorphic erythrocytes with its cut-off, so the clinician will be more alert to abnormalities that cause hematuria. Further research with larger samples and definite diagnosis with a kidney biopsy is needed to obtain more accurate results.

Keywords: Systemic lupus erythematosus, hematuria, dysmorphic erythrocytes, low condenser light microscope, phase contrast microscope, UF-500i

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a complex autoimmune and multi-system disease characterized by various clinical manifestations and the production of autoantibodies against a nuclear antigen. Kidney damage caused by SLE is called Lupus Nephritis (LN). Lupus nephritis is the most severe manifestation of SLE and increases patients mortality or risk of chronic renal failure.[1]

Incidence rates of SLE and LN ranged from 1.8–7.6 cases per 100,000 people. Systemic lupus erythematosus predominantly affects young females at productive age, mostly at 15–45 years. Comparison of incidence between females and males are 10:1. Almost 35%–50% of patients with SLE are accompanied by kidney disease at diagnosis, and more than 60% during monitoring.[2]

Prevalence of hematuria was 0.2%-16%, depending on the population of screening.[3] Hematuria is a meaningful clinical sign of the disease, and it requires further examination to find out the cause. Hematuria is the only manifestation of the kidney in the Lupus Activity Criteria Count (LACC) criteria among seven parameters associated with active SLE. Hematuria is also one of the parameters in the SLE Disease Activity Index (SLEDAI).[4]

Hematuria may be caused by a kidney stone, glomerular and tubular diseases, malignancy or infections of the kidneys and urinary tract, and rupture of capillary vessels. Dysmorphic erythrocytes are erythrocytes with an irregular shape of the membrane, are vesicular, or protrude on the membrane surface.[3] Yu *et al.* found 14 variations of dysmorphic erythrocyte shapes in their research.[5] The exact cause of dysmorphic erythrocytes has not been fully elucidated. It was hypothesized that they resulted from environmental changes around erythrocytes. Dysmorphic erythrocytes appear in the urine when physiological barriers of the glomerular are disrupted. This barrier consists of endothelium capillaries, the basal membrane of the glomerulus, and podocytes. When this barrier breaks, erythrocytes leak out to the tubules and follow urine

flow. When passing this barrier, erythrocytes change their form. The combination of mechanical damage when erythrocytes penetrate the barrier and the difference in osmotic pressure when erythrocytes enter the tubules will give force to the membrane of erythrocytes.[3,6]

Evaluation of erythrocyte morphology in urine has been widely used to determine the diagnosis of glomerular or non-glomerular disease diagnosis. Most previous studies used Phase Contrast Microscope (PCM).[7] Previous studies used a light microscope without staining to evaluate dysmorphic erythrocytes, and the results were similar to using a phase-contrast microscope. Research to determine the percentage of dysmorphic erythrocytes that support the diagnosis of hematuria caused by the kidneys was still varied between 14%-80%. The average rate of dysmorphic erythrocytes was 40% from current research.

Many techniques were developed to detect dysmorphic erythrocytes, namely PCM, light microscope with or without staining, electron microscope, and automatic urinalysis. Methods other than PCM and light microscope require a lot of equipment and time. Early detection of hematuria and determination between glomerular and non-glomerular disease is crucial to determine the management and further examination.[8]

The unavailability of PCM as a recommended instrument for dysmorphic erythrocyte evaluation causes this examination to be rarely performed. When providing high sensitivity and specificity, examination with a conventional light microscope may be used as a substitute for PCM in conducting dysmorphic erythrocyte examination. The use of automated urine analysis instruments will simplify the evaluation of dysmorphic erythrocytes and support the diagnosis of hematuria due to kidney disease.

This study aimed to determine the diagnostic value of dysmorphic erythrocytes in SLE patients with hematuria using a Low Condenser Light Microscope (LCLM), PCM, and UF-500i urine analyzer.

METHODS

This research was a cross-sectional study. Subjects were patients conducting urinalysis and urinary sediment examination using UF-500i in the Central Laboratory of Dr. Soetomo General Academic Hospital, Surabaya. Diagnosis of SLE based on SLICC criteria and determined by a clinician from internal medicine and Pediatric Department of Dr. Soetomo General Academic Hospital, Surabaya. Non-SLE are hematuria patients with another diagnosis. Dr. Soetomo General Academic Hospital Ethical Committee had approved this research with Number 1267/KEPK/VI/2019.

This study was conducted from May 2019 to July 2019. Subjects were all hematuria patients who performed urinalysis and urine sediment examination using UF-500i in the Central Laboratory of Dr. Soetomo General Academic Hospital. Percentage of dysmorphic erythrocytes determined by officers who were experts in assessing dysmorphic erythrocytes using LCLM and PCM.

Inclusion criteria in this study were patients with hematuria conducting urinalysis and urinary sediment examination with UF-500i. Patients with residual urine less than 10 mL were excluded.

Dysmorphic flagging was issued automatically from UF-500i. The percentage of dysmorphic erythrocytes was calculated using LCLM and PCM. All samples were fresh urine inspected in less than two hours since sample collection. All examinations were conducted at the Central Laboratory of Dr. Soetomo General Academic Hospital.

The technique of urine sediment in this study followed the standards of the Clinical and Laboratory Standard Institute (CLSI). Urine with 10–12 mL volume was centrifuged at 1500 RPM for 5 minutes. The supernatant was removed until the remaining 0.5–1 mL of urine at the base of the tube, then slowly homogenized. One drop of urine sediment was put on an object glass and closed with a cover glass carefully to prevent any bubbles, then read under the microscope.[9] Examples of normal and dysmorphic erythrocytes images are presented in Figure 1.

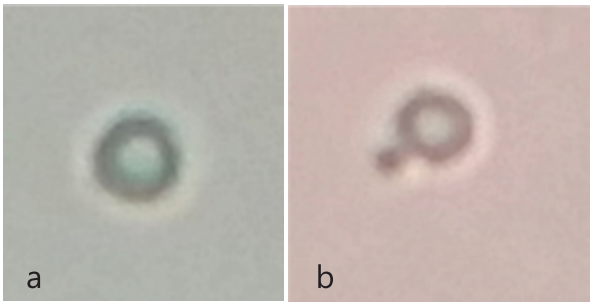

Figure 1. Normal erythrocytes (a) and dysmorphic erythrocytes (b)

Data were analyzed using SPSS 23.0. Normality tested using Kolmogorov-Smirnov test. The difference in the percentage of dysmorphic erythrocytes in LCLM and PCM was tested with the Wilcoxon Signed-rank test and the degree of agreement assessed with Cohen's Kappa coefficient. The sensitivity and specificity of each instrument analyzed based on the ROC curve are also used to determine cut-off. Results were statistically

significant when $p < 0.05$.

RESULTS AND DISCUSSIONS

Fifty-eight patients with hematuria who conducted urinalysis examinations were recruited in this study. Twenty-nine patients were diagnosed with SLE based on SLICC criteria. Thirty-seven patients were issued flagging dysmorphic on the examination of urinalysis using UF-500i. The patient's age varies from 1 to 65 years. Characteristics of the subject included age, gender, diagnosis, and the percentage of dysmorphic erythrocytes using LCLM and PCM were presented in Table 1. The majority of gender in this research subjects was female, with 33 patients and 25 male patients. Twenty-seven of them were diagnosed with SLE, and 31 others were diagnosed with non-SLE. The percentage of dysmorphic erythrocytes varies between 0-65% in LCLM and 0-67% in PCM. Median of dysmorphic erythrocytes in LCLM 7.5% and PCM 7%. Statistical analysis showed an abnormal distribution of sample data, so statistical analysis continued with a non-parametric test.

Table 1. Subject characteristics

Characteristics	Patients, n=58
Age (year)	
Median	16
Range	1-72
Gender	
Male	25
Female	33
Diagnosis	
SLE	27
Non-SLE	31
% Dysmorphic erythrocytes LCLM	
Median	7.5
Range	0-65
% Dysmorphic erythrocytes PCM	
Median	7
Range	0-67

The median number of dysmorphic erythrocytes in LCLM was 7.5% and at PCM 7%. Statistical analysis compared the percentage of dysmorphic erythrocytes in LCLM and PCM indicates the presence of statistically differences with $p < 0.001$ and a poor degree of agreement with Kappa coefficient 0.373 presented in Table 2.

Table 2. Difference test and degree of agreement

Difference Test and Degree of Agreement	n	p-value	Kappa Coefficient
% dysmorphic erythrocyte LCM and PCM	58	< 0.001	0.373

In this study cut-off for each instrument determine based on the best sensitivity and specificity obtained from the ROC curve (Fig. 2). Best cut-off for LCLM was 7.5% with sensitivity 70% and specificity 68% and for PCM 6.5% with sensitivity 74.1% and specificity 64.5%. Another cut-off for each instrument is presented in Table 3.

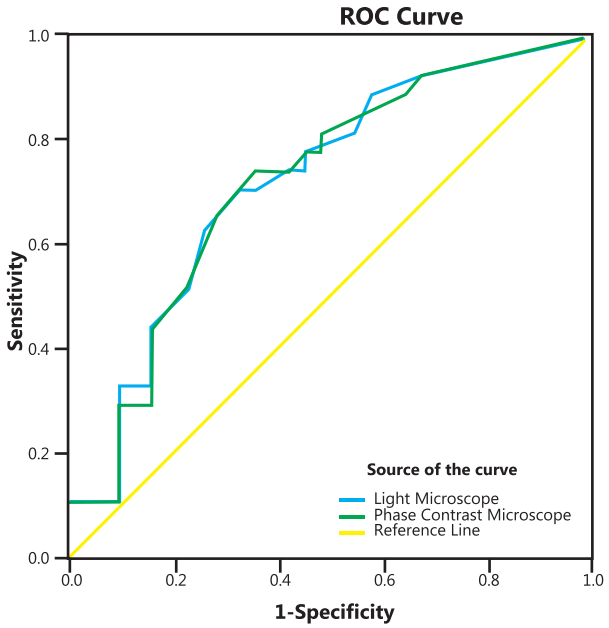

Figure 2. ROC analysis for LCLM and PCM

Table 3. Cut-off sensitivity and specificity of each instrument

Cut-off	LCLM		PCM	
	Sensitivity	Specificity	Sensitivity	Specificity
3.5%	78%	55%	78%	52%
6.5%	70%	65%	74%	65%
7.5%	70%	68%	67%	71%
13%	37%	84%	37%	84%
23%	26%	90%	26%	90%
40%	11%	97%	11%	94%

Research subjects were then divided into two groups that issue dysmorphic? flag, and who did not issue dysmorphic? flag on the Scattergram from UF-500i. Thirty-six patients issue dysmorphic? flag (+), 21 patients were diagnosed with SLE, while the remaining 15 patients were non-SLE. Twenty-two patients did not give dysmorphic? flag (-), six patients diagnosed with SLE and 16 others diagnosed non-SLE presented in Table 4. Based on the data, sensitivity, and specificity of dysmorphic? flagging was 78% and 52%, while PPV and NPV for dysmorphic? flagging was 58% and 73%.

Table 4. Dysmorphic? (+) and dysmorphic? (-) group based on the diagnosis of SLE and non-SLE

	SLE	Non-SLE	Total
Dysmorphic? (+)	21	15	36
Dysmorphic? (-)	6	16	22
Total	27	31	58

Many studies for the selection of the best microscope were used to evaluate the dysmorphic erythrocytes. Not only based on the numbers only, but also forms of cells found in the analysis and the best cut-off for the percentage of dysmorphic erythrocytes.[8] This study analyzed differences in the number of dysmorphic erythrocytes evaluated using LCLM and PCM, determining the best cut-off for each instrument.

Statistical analysis for the percentage of dysmorphic erythrocytes using LCLM and PCM indicate significant differences ($p < 0.001$). This result illustrates that LCLM is unable to replace PCM. This result was supported by a low agreement value based on the Kappa coefficient of 0.373. Thus, a light microscope with a low condenser couldn't replace the role of LCLM in evaluating the dysmorphic erythrocytes. Still, if determined specific cut-off for LCLM, this instrument may be valuable.

The best cut-off for LCLM in this research based on ROC curve analysis was 7.5%, with a sensitivity of 70% and specificity of 68%. The cut-off for PCM is 6.5%, with a sensitivity of 74% and specificity of 65%. This low cut-off might be because the research subjects were not all newly diagnosed patients, some of which were outpatients that have received prior therapy.

Research to determine the cut-off of the dysmorphic erythrocytes had various results. The most commonly used cut-off today is 40%.[10] In this study, if the 40% cut-off is used, the specificity will rise to 97% for LCLM and 94% for PCM, but the sensitivity will decrease drastically to 11% on both instruments.

The use of automated urinalysis is helpful in terms of time efficiency. However, in the evaluation of urine sediment, manual examination with a microscope is still regarded as the gold standard. Dysmorphic? flagging is one of the features of UF-500i to warn us about the abnormal form of the erythrocytes.

There has been no research linking dysmorphic? flagging and SLE. This research showed sensitivity and specificity of flagging dysmorphic? in SLE patients with hematuria of 78% and 52%, while PPV and NPV were 58% and 73%. These results may be due to a low sample count and more groups with diagnosis non-SLE.

The limitation of this study is a low number of samples, diagnosis without conducting kidney biopsy, and retrieval of subjects from patients with therapy.

CONCLUSIONS AND SUGGESTIONS

A low condenser light microscope may be considered a substitute for PCM for evaluating dysmorphic erythrocytes with its cut-off, so clinicians will be more alert to abnormalities that cause hematuria. Dysmorphic? flagging may be used as a warning sign but still need manual confirmation utilizing a microscope.

Further research with larger samples and definite diagnosis with a kidney biopsy is needed to obtain more accurate results.

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