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Soluble CD163 as a biomarker for renal involvment in systemic lupus erythematosus patients

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Abstract

Introduction: Urine-soluble CD163 (usCD163) is released from alternatively activated macrophages which play an important role in the pathogenesis of lupus nephritis. This study explored the role of usCD163 in patients with systemic lupus erythematosus (SLE). **Subject and methods:** usCD163 concentrations were measured cross-sectionally in 40 SLE patients and 20 apparently healthy volunteers, age and sex matched. Clinical and laboratory data were collected, a renal biopsy was obtained and SLE disease activity scores were calculated to assess the correlation with usCD163. **Results:** SLE patients with high usCD163 levels tended to have higher proteinuria, more pyuria and hematuria, higher levels of inflammatory markers, lower complement 3 (C3) levels, higher anti-double-stranded DNA antibody (anti-dsDNA Ab) levels, and higher disease activity scores ($p < 0.001$). usCD163 levels were significantly higher in patients with active lupus nephritis (LN) than in those with active extrarenal or inactive SLE and correlated with disease activity, and anti-dsDNA Ab levels. **Conclusions:** Urinary soluble CD163 reflects histologic inflammation in lupus nephritis and is a promising non-invasive biomarker for the assessment of renal disease in SLE patients.

Key words: Keywords: CD163, lupus nephritis, urine biomarker, renal pathology, activity index

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the production of autoantibodies and involvement of multiple organ systems. One of the leading causes of morbidity and mortality in SLE is lupus nephritis(LN), which is clinically evident in more than half of all SLE patients. Approximately 10–17% of LN patients will progress to end-stage renal disease (ESRD) (1).

Renal biopsy is the current gold standard for the diagnosis and classification of LN. However, the limited tissue obtained each time may not accurately reflect the complete spectrum of renal lesions in a given patient's kidneys due to sampling error; moreover, its invasiveness and attendant complications discourage repeated biopsy at patient follow-up. In contrast, urine samples can be easily obtained and are ideal for frequent monitoring. Non-invasive urinary biomarkers may emerge as an alternative method for LN assessment, as these markers are more convenient to assay, one day even at home, and allow repeated examinations (2).

CD163 is a marker for alternatively activated macrophages, which have been implicated in the pathogenesis of LN. While CD163C macrophages can only be observed and analyzed on tissue biopsies, soluble CD163 (sCD163), derived from the extracellular portion of CD163 when cleaved by metalloproteinases, can easily be measured in diverse body fluids, including serum, urine, synovial fluid and cerebrospinal fluid (3).

CD163+ macrophages are considered to be M2 cells with anti-inflammatory activity. Stimulation with interleukin (IL)-6, IL-10 or macrophage colony-stimulating factor (M-CSF) induces differentiation of naïve M0 macrophages into CD163+ cells. In both healthy and diseased individuals (4).

2. Patients and methods

This study included 40 individuals with SLE as group I (patients' group) who were subdivided according to the presence of LN into 2 subgroups: 20 SLE patients with LN (Ia) and 20 SLE patients without LN (Ib). A 20 age and sex-matching subjects who were apparently healthy served as controls (group II). Patients were recruited from the outpatients' clinic and the inpatients' department of Rheumatology, Rehabilitation and Physical Medicine at Benha University Hospitals, Egypt. All patients met the ACR/EULAR 2019 revised SLE classification criteria (5). Patients with autoimmune diseases other than SLE or those younger than 18 years of age did not participate in the study. This study has been approved by the local Ethics Committee of Benha University, Egypt. An informed consent was provided by all patients. According to the total clinical SLEDAI score and the rSLEDAI index patients were categorized into 4 groups; 12 patients with active LN (renal SLEDAI score ≥ 4), 12 patients with active non-renal SLE (total clinical SLEDAI score ≥ 1, but rSLEDAI=0), 8 patients with inactive LN and a history of LN (total SLEDAI score = 0 and rSLEDAI = 0) and 8 patients with an inactive non-renal SLE (total clinical SLEDAI score = 0).

A full medical history was taken and a thorough clinical examination was done. SLE disease activity was assessed using the SLEDAI and renal activity was assessed using the renal domain scores (rSLEDAI) (6). The following laboratory tests were conducted: Acomplete blood count (CBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), complement proteins 3 and 4 (C3 and C4), antinuclear (ANAs), anti-double-stranded antibodies (anti-dsDNAs), renal function tests and urinary sCD163 level. Levels of urinary sCD163 was assessed using a commercial human enzyme-linked immunosorbent assay (ELISA) kit (Bioassay technology laboratories , Shanghahai Korain Biotech, Cat. A renal biopsy was

done when indicated (proteinuria ≥ 500 mg/24 hr, persistent hematuria or pyuria after exclusion of other potential causes or in case of unexplained renal insufficiency with normal urinalysis) (7). The biopsy was obtained by radiology consultant under computed tomography "CT" guidance using a true cut needle biopsy at radiology department at Benha University Hospitals. The predominant histopathological feature was classified according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification system (8).

Statistical analysis

Data were presented as mean and standard deviation (SD), and the range of values were reported. The following tests were applied: Student's t-test to assess the statistical significance of the difference between the two study group means, the Mann–Whitney test to assess the statistical significance of the difference of a non-parametric variable between two study groups, the 95 % confidence interval (CI) was to estimate the precision of the odds ratio (OR) and Operating characteristic curve (ROC) analysis, and the corresponding area under the curve (AUC; range 0–1) was calculated. A *p* value is considered significant if <0.05 at confidence interval 95%. Sample size was calculated by Stata Corp. 2021. Stata Statistical Software: Release 17. College Station, TX: Stata Corp LLC.Using t test model, and receiver operating characteristic (ROC) curve model; required minimal sample size is 60 subjects (40 SLE cases and 20 healthy control subjects), using α error 5% and a power of 80%. All analyses were performed using the SPSS software version 22 (IBM Corp., Armonk, New York, USA).

3.Results

This study included 40 patients with SLE (31 females (77.5%) and 9 males (22.5%)). The mean age of the patients was 28.4±4.803 years and their disease duration was 3.4±1.87 and 5.5 ± 3.05yrs years for SLE patients with and without LN respectively. Patients and controls were matched for age (p>0.05) and sex (p>0.05).

The mean usCD163 level was significantly increased in SLE patients compared to healthy controls (p < 0.001). Figure (1)

The mean usCD163 level was significantly increased in Lupus nephritis group compared to Lupus without nephritis group (p<0.001). Figure (2).

Regarding to usCD163 level there was highly statistically significant difference between the active and inactive SLE with LN (p<0.001), and statistically insignificant difference between the active and inactive SLE patients without LN (p=0.38) Figure (3).

The usCD163 was higher in class III , IV (prolferative LN) than class V (non proliferative LN) and there was highly statistically significant difference (p<0.001) among them and was lower in patients with negative biopsy.

there were statistically significant positive correlations of usCD163 with proteinuria, urinary RBCs, urinary pus cells, s. creatinine, Bl urea, SLEDAI **(Figure 4a)** , rSLEDAI, Anti dsDNA value (all p<0.001), Activity index (p<0.006) **(Figure 4b),** Chronicity index (p<0.001), disease duration (p=0.014), Hb (p=0.031), CRP (p=0.032), statistically significant negative correlation to s.albumin (p<0.001) **(Figure 4c)** and C3 **(Figure 4d)** (p<0.001) and C4 (p=0.01).

there were highly statistically significant associations between usCD163 and malar rash, arthritis, nephritis, pericarditis, pleurisy ,anemia, leucopenia (p<0.001) and statistically significant associations between usCD163 and thrombocytopenia (p=0.04).

the predictive value of usCD163 was assessed using Receiver Operating Characteristic (ROC) curve. Area Under the Curve of uCD163 = 1.0 (95 % C.I = 1.00-1.00), Sensitivity and specificity values were 100 % & 100 % respectively**(Figure 5)**.

Table (1) comparison between the SLE and control groups regarding their demographic characteristics

Study groups	Systemic Lupus disease N.=40		Control N.=20			
variable	Mean or N.	±SD or %	Mean or N.	±SD or %	Statistical test	P value
Age (years) (mean ±SD)	28.40	4.803	27.2	5.146	Student t test = 0.891	0.377
Sex					Chi square test = 1.337	0.521
Female	31	77.5%	15	75%		
Male	9	22.5%	5	25%		

Table (2) comparison between LN and lupus without nephritis groups regarding their demographic characteristics and disease duration

Study groups	Lupus nephritis N.=20		Lupus without nephritis N.=20		Statistical test	P value
variable	Mean or N.	±SD or %	Mean or N.	±SD or %		
Age (years) (mean ±SD)	27.4	4.806	29.4	4.706	Student t test = 1.33	0.192
Sex					Chi square test =	
Female	14	70%	17	85%	1.29	0.256
Male	6	30%	3	15%		
Disease duration (years) (mean ±SD)	3.4	1.87	5.6	3.05	Student t test= 2.748	0.01 (S)

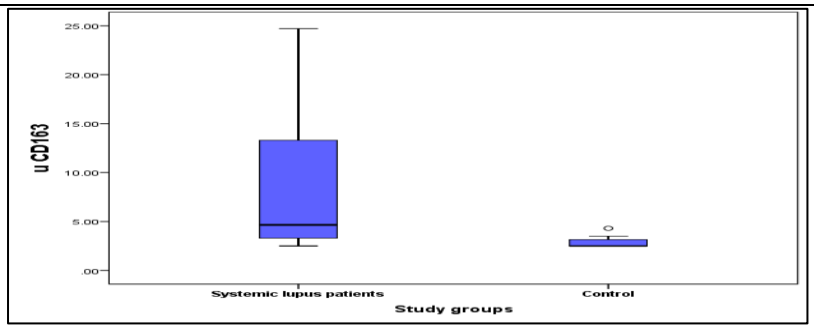


Figure (1): comparison of usCD163 level between SLE and control groups

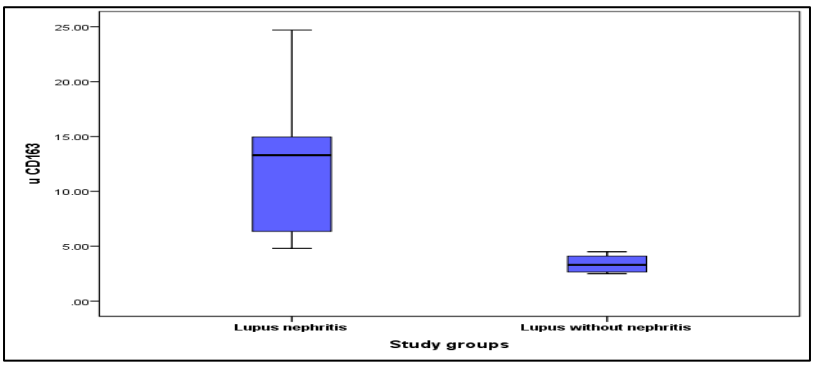


Figure (2): comparison of usCD163 level between LN and lupus without nephritis groups

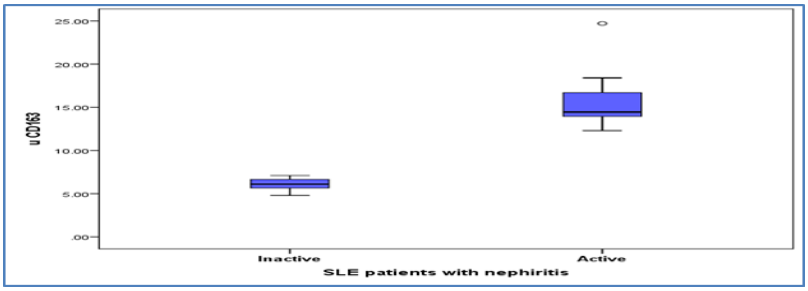


Figure (3): comparison of usCD163 level between active and inactive LN groups

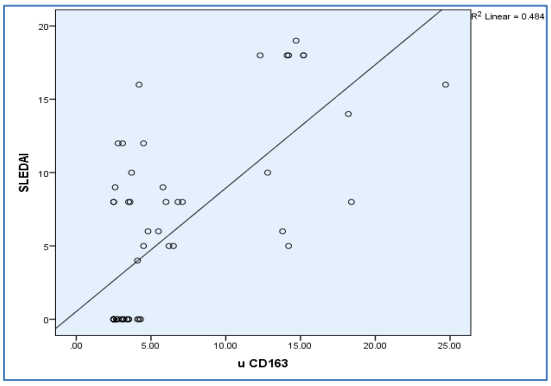
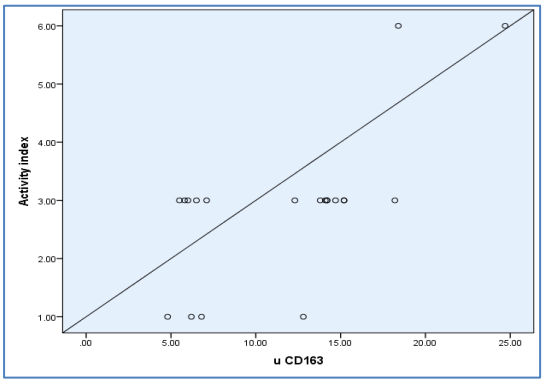


Figure (4a): Correlation between usCD163 level and SLEDAI index



Figure(4b):usCD163 level and activity

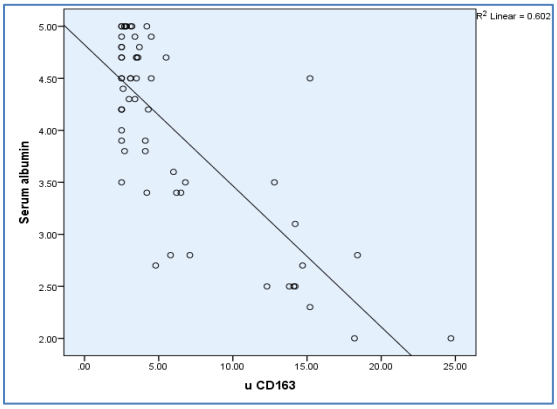


Figure (4c): usCD163 and serum albumin

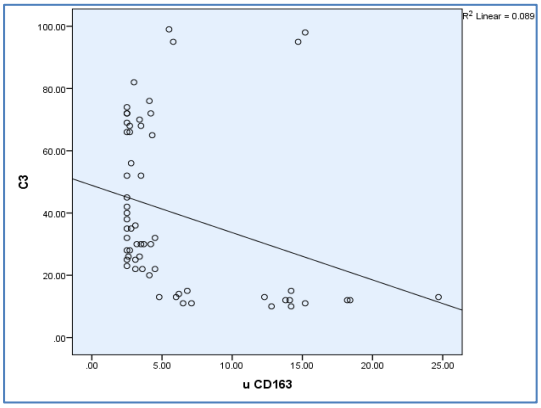


Figure (4d): Correlation between usCD163 level and C3

4.Discussion

The clinical manifestations of LN are variable, ranging from asymptomatic proteinuria to rapidly progressive nephropathy and despite the advancements in understanding of the pathophysiology of LN and the development of treatment strategies, only 50–70% of patients achieve remission, therefore it is important to screen all SLE patients for LN (9).

Conventional biomarkers, including C3, C4 and anti-dsDNA antibody have been classicaly used to evaluate general disease activity in SLE. However, they do not predict or correlate well with LN or disease flares. Urinary biomarkers have now emerged as a potential tool for evaluating LN and potential treatment targets, as these proteins may arise directly from the inflamed kidneys (10).

Through this study, we have demonstrated that sCD163 in urine from patients with active LN is significantly higher as compared with the urinary levels in patients with active disease without nephritis, inactive lupus with or without LN and healthy individuals.

These results confirmed by Mejia-Vilet and colleagues (11) , Huang and colleagues (12) who stated that usCD163 levels were significantly increased in active LN patients compared to active SLE patients without renal involvement and inactive SLE patients. Moreover, results of studies conducted by O'Reilly and colleagues(13), Zhang and colleagues (1), corroborate our finding demonstrating that sCD163 increases both in plasma and urine during active lupus nephritis compared to inactive SLE patients with LN, but unlike our results Nishino and colleagues (14), Nakayama and colleagues (15) who showed that Plasma soluble CD163 levels were higher in active SLE but did not differ between active nephritis and active non-renal which suggests that plasma levels reflect only systemic inflammation and not renal inflammation.

Our study found that usCD163 was significantly higher in class III, IV (proliferative LN) compared to class V (non-proliferative LN) ($p<0.001$) and was lower in patients with negative biopsy so we were in agreement with Zhang and colleagues (1) who found that urine sCD163 was significantly increased in patients with proliferative LN when compared with non- proliferative LN in 137 LN patients.

Also these results concurred with Endo and colleagues (16) and Olmes and colleagues (17) who found that among patients with LN, class III and IV LN patients showed increased usCD163 levels relative to class V patients and the glomerular accumulation of CD163+ cells was high in LN class IV and minimal in stage V while plasma CD163 levels were not associated with ISN/RPS class suggesting that increased usCD163 reflect renal activity but unlikely to be the consequence of enhanced systemic activation of macrophages.

Our study revealed that urine sCD163 highly significantly correlated with conventional parameters including proteinuria, urinary RBCs, urinary Pus cells, s.creatinine, blood urea (all p<0.001).This agreed with the finding of Gupta and colleagues (18) who demonestrared that urinary soluble CD163 correlated with different disease activity parameters in active nephritis group such as proteinuria, urinary RBCs, urinary pus cells, s.creatinine whereas plasma soluble CD163 did not.

Our work revealed significant correlations of urine soluble CD163 with SLEDAI, rSLEDAI, C3, C4, anti-dsDNA antibody and activity index (AI) of renal pathology (all P < 0.01). This outcome parallels the finding of Zhang and colleagues (1) who found that usCD163 positively correlated anti-dsDNA abs, Olmes and colleagues (17) who found that usCD163 positively correlated with ESR, proteinuria, urine protein to creatinine ratio and anti-dsDNA abs, and Huang and colleagues (12) who found usCD163 levels were correlated with lower estimated glomerular filtration rate, higher urine protein/creatinine ratio (UPCR), more pyuria and hematuria, higher levels of inflammatory markers, higher rates of anemia, neutropenia, and lymphopenia, lower C3 levels, higher anti-dsDNA Ab levels, and higher disease activity scores.

It is worth to be notrd that usCD163 in some studies not only has significant correlations with renal activity parameters but also has significant correlations with non-renal activity parameters but this may be explained by the presence of extra-renal manifestations in LN pts(12).

Our study showed that the level of usCD163 showed a highly statistically significant difference between active LN and inactive LN and the cut-off value for discrimination between them was >4.65 ng/mg with a sensitivity of 100% and specificity of 100 %.These results were accorded with Gupta and colleagues (18), Zhang and colleagues (1), Huang and colleagues (12), who showed that urinary soluble CD163 can differentiate between lupus patients who have active disease with nephritis from ones who have active disease without nephritis. Its levels correlate with conventional disease activity parameters and decrease as the disease activity decreases with treatment. Thus, it is a good marker of lupus nephritis activity.

Also our results agreed with Mejia-Vilet and colleagues (11) that emphasized that in patients with LN, usCD163 levels change throughout the disease and treatment process. Initially, usCD163 levels increase before a flare-up of LN, reach their peak during the flare-up, and then decrease as treatment is administered, particularly in patients who respond well to therapy. By the end of the initial treatment phase, usCD163 levels show improvement. Measuring usCD163 levels at the end of the intensive immunosuppressive phase of LN therapy can be helpful in making treatment decisions. It can accurately identify patients who are likely to achieve complete remission, as indicated by a high sensitivity and specificity of this prediction. In comparison, proteinuria, another marker used in LN, improves more slowly and takes longer to reach its lowest point, typically during the maintenance phase of treatment.

In addition, Dekkema and colleagues (19) in a study on ANCA associated vasculitis patients found that usCD163 differentiated active from inactive renal vasculitis and has been previously shown to increase in several active glomerular diseases. In ANCA associated vasculitis (AAV) a cutoff of 300–350 ng/mmol with >70% sensitivity and >94% specificity.

Our results didn't agree with Endo and colleagues (16) who documented that the usCD163 is not specific for LN and its levels are elevated in several other glomerular diseases Therefore, although usCD163 is useful to identify the inflammatory activity of LN, it does not differentiate between active LN and other inflammatory glomerular diseases.

Limitations of the current study were, the small sample size, variations in the clinical presentation of patients, the use of different types of therapy, different laboratory techniques and the initial renal histopathology cannot represent the subsequent disease activity of LN during the follow-up period. To qualify usCD163 as a response biomarker and to demonstrate its superiority over proteinuria and eGFR, a prospective follow-up study comparing renal histologic response and usCD163 will be needed. Moreover, serial usCD163 measurements and long-term follow-up of renal condition were not performed, so further multicenter studies should be carried out.

In conclusion, Urinary CD163 reflects histologic inflammation in lupus nephritis and is a promising non-invasive biomarker for the assessment of renal disease in SLE patients

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