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Original article

Serum myeloperoxidase level as A marker of activity in patients with systemic lupus erythematosus with lupus nephritis class III and IV

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Abstract

Aim: The aim of the study was to assess serum levels of MPO in lupus nephritis class III and IV patients and their relationship with disease activity parameters.**Methods:** Forty patients with Systemic Lupus Erythematosus (SLE) and forty healthy controls were investigated in this study diagnosed according to European league against rheumatism &American colleague of rheumatology 2019(EULAR/ ACR) Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and laboratory parameters, including erythrocyte sedimentation rate, ANA, anti-ds DNA antibody, complement 3, complement 4 levels and CRP were analyzed. The serum myeloperoxidase (MPO) was determined using enzyme-linked immunosorbent assay. **Results:** The mean Anti ds DNA, complement 3&4, ESR, and ANA titer were significantly higher among cases than controls. The mean myeloperoxidase marker was

significantly higher among cases than controls (p value <0.001*). Serum myeloperoxidase had a significant role in prediction of lupus nephritis at a cut off more than 178.5; it can predict the LN with 80% sensitivity, 50% specificity, 61.5% PPV and 71.4% NPV. The A/C ratio seemed to be highly predictor for LN than the serum myeloperoxidase as at a cut off more than 30, it can predict the LN with 100% sensitivity, 97.5% specificity, 97.6% PPV and 100% NPV. **Conclusion:** This study suggests a good correlation between MPO and SLE disease activity index indicating its direct involvement in inflammatory conditions associated with disease.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic, multi-factorial inflammatory autoimmune disease that primarily affects women between the ages of early adulthood and mid-adulthood. It is characterized by the production of a wide range of autoantibodies and immune complex deposition, which can damage multiple organs **(1).**

The inflow of pro-inflammatory cytokines mediates an inflammatory response. Various acute-phase reactant proteins, such as high sensitivity C-reactive proteins (hs-CRPs), lectin proteins, hepcidin, etc., are produced as a result of these cytokines, and these proteins are recognised as inflammatory indicators **(2).**

SLE has a complicated pathophysiology that is linked to excessive T and B cell activation, impairment of apoptosis, and insufficient immune complex clearance. Overactive B cells produce too many autoantibodies, which bind with chromatin to form immunological complexes and cause inflammation **(3)**

Systemic lupus erythematosus disease activity index 2000 (SLEDAI-2K), British Isles lupus as assessment group (BILAG), and laboratory parameters C3 and C4 complement components, anti-C1q antibodies, and anti-double stranded DNA antibodies (Anti-dsDNA titer) are used to assess SLE activity in clinical practice **(4).**

In particular, anti-MPO antibodies will be covered. According to one research, SLE or a drug-induced condition that is similar to SLE, drug-induced vasculitis or nephritis, and idiopathic vasculitis are all associated with high titers of anti-MPO antibodies **(5).**

Anti-MPO antibodies are thought to play a part in processes that activate neutrophils, causing neutrophil extracellular traps (NET) to develop and tissue damage in either drug-induced lupus or idiopathic SLE. Interestingly, a research found that MPO plasma levels were significantly greater in SLE patients than in healthy individuals, despite the fact that this did not correspond with the severity of the condition **(6).**

2. Aim of the Study:

This study aimed to assess the serum MPO and assess its correlation with lupus nephritis activity in class III, IV by SLEDAI as disease activity score.

3. Subjects and Methods:

This case control study was conducted on 40 clinically diagnosed SLE patients (23 females & 17 males) who were recruited from internal medicine Department (clinical immunology unit) of Beni Suef university hospital in a period from March 2020 to September 2020.

The patients were diagnosed according to 2019 EULAR\ACR classification criteria for systemic lupus erythematosus (7) & forty healthy adults served as a control group.

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Inclusion criteria:

Forty SLE patients and forty healthy controls. Patients were diagnosed as lupus nephritis class III or IV with renal biopsy with age ranged from 13 to 45 years old.

Exclusion criteria:

Patients less than 16 or older than 45 years with end stage renal disease were excluded.

Methods:

All patients were subjected to full clinical and laboratory investigations. A sheet was formulated and applied to all patients subjected to this study. Personal history as age, sex., residence and menstrual history. Complaint and present history of the disease as age of onset, disease course and duration. Constitutional, mucocutaneous symptoms, articular symptoms, muscular symptoms, cardiopulmonary symptoms, gastrointestinal symptoms, genitourinary symptoms, obstetric history, comorbidities and drug history.

In addition, full clinical examination as BMI, vital signs, eyes and mouth examination, skin examination, muscle examination, articular examination, chest examination, cardiac examination, neurological examination, abdominal examination and examination of the lymph nodes and peripheral pulses.

Laboratory Investigations:

Routine laboratory investigations included CBC, ESR, ANA, Complement 3 and 4, 24 hours urinary proteins, serum urea and creatinine, Anti-dsDNA, complete urine analysis, lipid profile (cholesterol, triglycerides, LDL, HDL), and serum uric acid. Renal Biopsy was done for eligible patients according to P/ C ratio and classified according to the classification of lupus nephritis by the International Society of Nephrology/Renal Pathology Society (ISN/RPS) (8).

Special laboratory tests:

Detection of serum MPO by ELISA technique.

Principle of the assay:

The kit measures the amount of human MPO in a sample by coating micro titer plate wells with purified human MPO antibody, creating solid-phase antibody, adding MPO to the wells, and combining the antibody with goat anti-human that has been HRP labelled to create an antibody-antigen-enzyme-antibody complex. Finally, after thorough washing, adding TMB substrate solution causes the TMB substrate to turn blue. A sulphuric acid solution is added to stop the HRP enzyme-catalyzed process, and the colour change is assessed spectrophotometrically at a wavelength of 450 nm. The O.D. of the samples is then compared to the standard curve to determine the concentration of MPO in the samples.

Specimen requirements:

Centrifuged at a speed of 2000–3000 rpm for 20 min after allowing serum to coagulate at room temperature for 10–20 min. Remove supernatant and centrifuge once more if precipitation forms. Samples were kept without undergoing multiple freeze-thaw cycles at -20°C or -80°C.

Radiological Investigations: if indicated in the form of Pelvi abdominal ultrasound.

Statistical analysis:

Analysis of data was performed using SPSS v. 25 (Statistical Package for Social science) for Windows. Description of quantitative variables was in the form of mean, standard deviation (SD), minimum, maximum and median. Description of qualitative variables

was in the form of numbers (No.) and percent's (%). Mann-Whitney U test was used to compare between two groups regarding scale variables while independent T test was used to compare between groups regarding normally distributed variables. ROC curve was used to predict optimal cut off in marker and A/C ratio in prediction of SLE nephropathy. Pearson correlation was used to correlate between normally distributed variables. The significance of the results was assessed in the form of P-value that was differentiated into: non-significant when P-value > 0.05 and significant when P-value ≤ 0.05

Ethical considerations:

The study protocol was approved by the research ethics committee of Faculty of Medicine of Beni-Suef University number FMBSUREC/03012021/Ali. An informed consent was obtained from the participants and all data was anonymous and confidential.

4. Results:

Table (1) showed that there was no significant difference between cases and controls regarding their age, sex, hypertension and diabetes mellitus distribution. There were no cases of diabetes among both groups. The mean systolic and diastolic blood pressure were significantly higher among cases than controls but both groups were with normotensive blood pressure.

Table (1) Age and sex distribution, comorbidities of medical importance and blood pressure among the studied groups:

Items	**Cases (no=40)**	**Controls (no=40)**	**P-value**
Age (mean±SD)	33.8±6.6	35.7±9.2	0.279
Sex Females Males	 23(57.5%) 17(42.5%)	 25(62.5%) 15(37.5%)	0.648
HTN	4(10.0%)	0(0.0%)	0.116
DM	0(0%)	0(0%)	---
Systolic BP	121.7±15.6 mmhg	109.3±7.6 mmhg	<0.001*
Diastolic BP	78.5±6.6 mmhg	69.5±7.1 mmhg	<0.001*

**P-value is significant*

Table (2) showed that the mean Albumin/Creatinine ratio was significantly higher among cases than controls in contrast to the hemoglobin; it was significantly higher among controls than cases. There was no significant difference between cases and controls regarding the level of Sodium and Potassium, and Creatinine. The mean Anti DNA , ESR, and ANA titer were significantly higher among cases than controls, but C3 and C4 were significantly higher in controls than cases.

Table (2) Laboratory and inflammatory markers distribution among the studied groups:

Items	Cases (no=40)	Controls (no=40)	P-value
Na	139.6±3.1mEq/L	139.4±3.1 mEq/L	0.803
K	4.8±5.5 mEq/L (median=4)	4.7±5 mEq/L (median=3.9)	0.905
Create.	1.2±0.5 mg/dl	1.2±0.3 mg/dl	0.769
Hb	10.1±1.4 gm/dl	11.9±1.1 gm/dl	<0.001*
A/C ratio	2688.7±1967.2mg/dl	22.3±6.3 mg/dl	<0.001*
Anti DNA	172.6±278.8 (median=96)	9.4±3.9 (median=9)	<0.001*
C3	69.6±27.7 mg/dl	132.9±20.2 mg/dl	<0.001*
C4	15.2±7.2 mg/dl	23.4±6.9 mg/dl	<0.001*
ESR	76.2±24.2mm/hr	15.8±4.9 mm/hr	<0.001*
ANA titer	1/211.4±218.5	1/20±0	<0.001*

**P-value is significant*

This study showed that the mean SLEDAI score was 37.6±14.4 and ranged from 14 to 75 with median 40. This table showed that the mean myeloperoxidase marker was significantly higher among cases than controls (Table 3)

Table (3) Comparison between cases and controls regarding the level of serum myeloperoxidase marker:

Items	Cases (no=40)	Controls (no=40)	P-value
Myeloperoxidase	243±79	187.4±70.5	0.001*

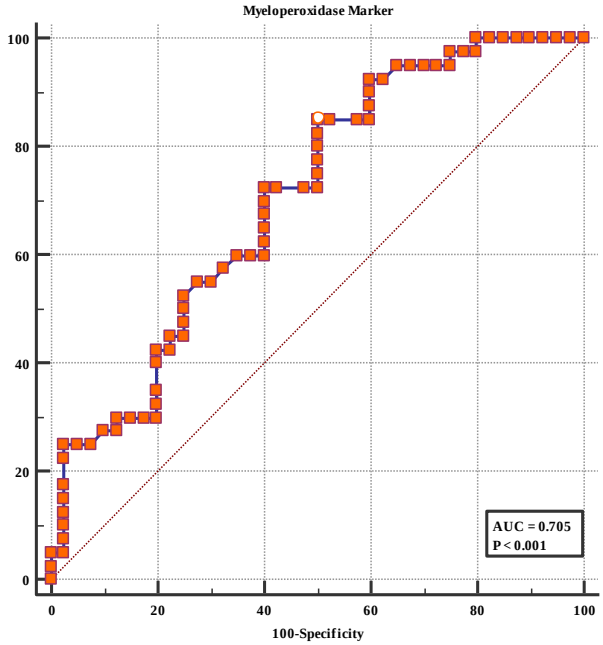

Figure (1) Receiver Operating Characteristic curve for prediction of lupus nephritis using serum Myeloperoxidase

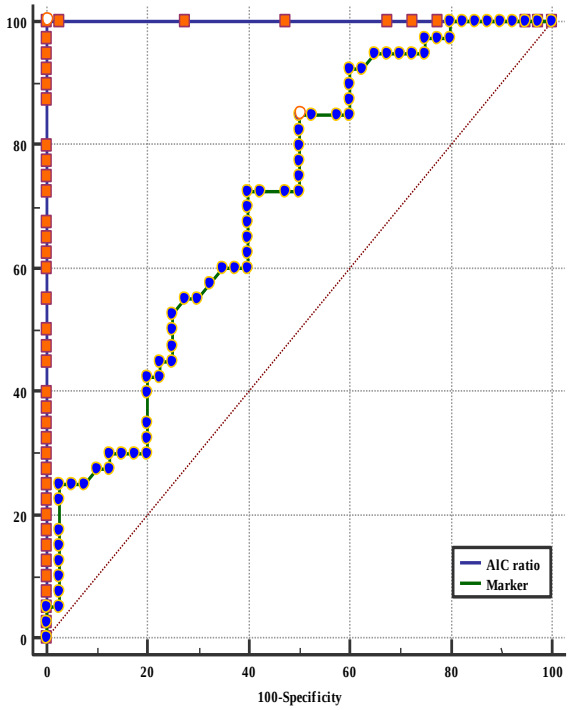

Figure (2): Receiver Operating Characteristic curve for prediction of lupus nephritis using serum Myeloperoxidase compared to A/C ratio.

Table (4) Sensitivity, specificity, PPV and NPV of serum Myeloperoxidase in detection of lupus nephritis compared to A/C ratio:

Items	**Myeloperoxidase**	**A/C ratio**
P-value	0.001	<0.001*
Cut off	>178.5	>30
AUC	0.705	1.000
Sensitivity (95%CI)	80 (64.4 - 90.9)	100(91.2 – 100)
Specificity	50 (33.8 - 66.2)	97.5(86.8 - 99.9)
PPV	61.5(53.1 - 69.3)	97.6(85.2 - 99.6)
NPV	71.4(55.6 - 83.3)	100(91.2 – 100)

Table (4) and figure (1,2) showed that the serum myeloperoxidase had a significant role in prediction of lupus nephritis at a cut off more than 178.5, it can predict the LN with 80% sensitivity, 50% specificity, 61.5% PPV and 71.4% NPV. The A/C ratio seemed to be highly predictor for LN than the serum myeloperoxidase as at a cut off more than 30, it can predict the LN with 100% sensitivity, 97.5% specificity, 97.6% PPV and 100% NPV.

Table (5) showed that there was no significant linear correlation between serum level of myeloperoxidase and different parameters except ESR and SLEDAI score, ESR had a moderate linear positive correlation with the marker level. SLEDAI score had a strong positive linear correlation with myeloperoxidase.

Table (5) Correlation between the serum Myeloperoxidase and different parameters among cases

Independent variables among cases		**Myeloperoxidase**
Age	**R**	0.172
	P-value	0.287
BP systolic	**R**	0.237
	P-value	0.140
BP diastolic	**R**	0.208
	P-value	0.199
Na	**R**	0.010
	P-value	0.951
K	**R**	-0.022
	P-value	0.893
Creatinine	**R**	0.049
	P-value	0.763
HB%	**R**	-0.033
	P-value	0.842
A/C ratio	**R**	0.096
	P-value	0.556
anti dDNA	**R**	-0.012
	P-value	0.940
C3	**R**	-0.006
	P-value	0.972
C4	**R**	0.139
	P-value	0.393
ESR	**R**	0.408**
	P-value	0.009
ANA titer	**R**	-0.068
	P-value	0.675
SLEDAI score	**R**	0.610**
	P-value	<0.001

**P-value is significant*

5. Discussion:

SLE is a potentially serious autoimmune condition that exhibits racial and ethnic differences in incidence, prevalence, disease activity, and prognosis **(9).**

Through the local activation of the complement system, which results in cellular proliferation, accumulated immune complexes cause inflammation. When compared to LN, pauci-immune glomerulonephritis (GN) differs in that glomerular necrosis and crescent development take place in the absence of considerable cellular proliferation and glomerular immune-complex deposits are only sometimes seen **(10).**

The pathophysiology of this kind of glomerular damage is directly linked to antineutrophil cytoplasmic antibodies (ANCAs), which are considered to activate cytokine-primed neutrophils and monocytes that display the ANCA antigens proteinase 3 and myeloperoxidase (MPO) on their surface **(11).**

Patients with systemic lupus erythematosus (SLE) may develop autoimmunity due to inadequate clearance of dead cells **(12).**

Prior to the discovery of neutrophil extracellular traps, apoptotic cells were long thought to be the primary source of autoantigens (NETs). NETs are structures made of neutrophil DNA that are embellished with antimicrobial proteins. Neutrophil DNA is often expelled in response to various stimuli, most commonly cytokines and microbial products (13). MPO is a fundamental component of NETs (14).

The results of this investigation demonstrated that cases had considerably greater mean myeloperoxidase markers than controls. This finding is consistent with a prior work by Hirai et al. (2008) who discovered that MPO-ANCA, the immune complex deposition, may have aggravated the endothelial damage, resulting in particularly severe histological characteristics and a challenging clinical course in SLE patients **(15).**

In addition, our result is agreed with a recent Egyptian study that showed that there was a positive correlation between MPO-ANCA and SLEDAI, as well as with class IV LN and MPO-ANCA level was significantly correlated with SLEDAI and inflammatory markers, as ESR in our results **(16).**

On contrary to our results, the literature, results related to MPO concentration in SLE are conflicting. As Morgan et al. found decreased MPO levels in patients with SLE **(17).**

Regarding the predictivity of MPO in lupus nephritis, our case control study that showed that the serum myeloperoxidase had a

significant role in prediction of lupus nephritis at a cut off more than 178.5, it can predict the LN with 80% sensitivity, 50% specificity, 61.5% PPV and 71.4% NPV. This was agreed with a previous study that revealed that the sensitivity and specificity of MPO-ANCA were 81.3% and 99.8%, respectively, in discriminating LN from systemic lupus without nephritis **(16).**

Additionally, Olson et al., 2017's retrospective case-control study, which compared MPO-ANCA levels in longitudinal pre-diagnostic serum samples from 23 biopsy-confirmed patients with proliferative lupus nephritis (PLN) to identified age, sex, race, and age of serum matched healthy and SLE without LN disease controls, supports our findings. They found that a higher proportion of PLN patients had prediagnostic M Subclinical dsDNA ab and the early onset of MPO-ANCA imply that it may directly contribute to PLN pathogenicity **(18).**

Li et al. found that MPO-ANCA-positive LN patients had significantly higher serum creatinine (109.6 mol/l vs. 74.3 mol/l, p = 0.02), lower titers of antinuclear antibodies (ANA) (128 vs. 256, p = 0.01), and higher serum concentrations of C3 and C4 (0.54 g/l vs. 0.36 g/l, The baseline renal function of the MPO-ANCA-positive LN patients was more significantly compromised. Patients with MPO-ANCA-positive LN had more severe chronic pathological alterations, such as interstitial fibrosis and tubular atrophy, on renal specimens **(19).**

That was in contrast with our study that showed that that ESR had a moderate linear positive correlation with the marker level. SLEDAI score had a strong positive linear correlation with myeloperoxidase.

The current study results is agreed with a previous one that found a significantly lower hemoglobin level (P=0.015), higher value of serum creatinine (P<0.001), lower level of creatinine clearance rate (P<0.001), and lower ratio of positive serum anti-ribonucleoprotein antibody (P=0.044) in patients with crescentic glomerulonephritis **(20).** This study was in accordance with our study that showed that the hemoglobin was significantly higher among controls than cases and in contrast with our study that showed that There was no significant difference between cases and controls regarding the level of Sodium and Potassium, and creatinine which may be due to most of our cases were recently discovered and there is no progression of renal condition.

6. Conclusion and recommendations:

In conclusion, our study concluded that serum MPO level was significantly high in patients with active lupus nephritis class III & IV and significantly correlated with lupus nephritis activity in class III, IV by SLEDAI score as disease activity score. This study recommends future large multicentric studies with larger sample size to ensure the external validity of the results.

7. References:

1. Somers, E. C., Marder, W., Cagnoli, P., Lewis, E. E., DeGuire, P., Gordon, C., ... & McCune, W. J. (2014). Population-based incidence and prevalence of systemic lupus erythematosus: the Michigan Lupus Epidemiology and Surveillance program. Arthritis & rheumatology, 66(2), 369-378.
2. Sproston, N. R., & Ashworth, J. J. (2018). Role of C-reactive protein at sites of inflammation and infection. Front Immunol. 2018; 9: 754. URL: https://www. frontiersin. org/articles/10.3389/fimmu.
3. Tsokos, G. C., Lo, M. S., Reis, P. C., & Sullivan, K. E. (2016). New insights into the immunopathogenesis of systemic lupus erythematosus. Nature Reviews Rheumatology, 12(12), 716-730.
4. Touma, Z., Gladman, D. D., Ibañez, D., & Urowitz, M. B. (2011). Development and initial validation of the systemic lupus erythematosus disease activity index 2000 responder index 50. The Journal of rheumatology, 38(2), 275-284.
5. Cambridge, G., Wallace, H., Bernstein, R. M., & Leaker, B. (1994). Autoantibodies to myeloperoxidase in idiopathic and drug-induced systemic lupus erythematosus and vasculitis. Rheumatology, 33(2), 109-114.
6. Telles, R. W., Ferreira, G. A., Silva, N. P. D., & Sato, E. I. (2010). Increased plasma myeloperoxidase levels in systemic lupus erythematosus. Rheumatology international, 30(6), 779-784.
7. Aringer, M., Costenbader, K., Daikh, D., Brinks, R., Mosca, M., Ramsey-Goldman, R., ... & Johnson, S. R. (2019). 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. Arthritis & rheumatology, 71(9), 1400-1412.
8. Weening, J. J., D'agati, V. D., Schwartz, M. M., Seshan, S. V., Alpers, C. E., Appel, G. B., ... & Nagata, M. (2004). The classification of glomerulonephritis in systemic lupus erythematosus

revisited. Kidney international, 65(2), 521-530.

9. Mok C, Penn H, Chan K, Tse SM, Langman LJ , Jannetto PJ. 2016.Hydroxychloroquine serum concentrations and flares of systemic lupus erythematosus: a longitudinal cohort analysis. Arthritis Care Res. 68(9):1295–302.
10. Nasr, S. H., D'Agati, V. D., Park, H. R., Sterman, P. L., Goyzueta, J. D., Dressler, R. M., ... & Markowitz, G. S. (2008). Necrotizing and crescentic lupus nephritis with antineutrophil cytoplasmic antibody seropositivity. Clinical Journal of the American Society of Nephrology, 3(3), 682-690.
11. Jennette, J. C., Falk, R. J., Hu, P., & Xiao, H. (2013). Pathogenesis of antineutrophil cytoplasmic autoantibody–associated small-vessel vasculitis. Annual review of pathology, 8, 139.
12. Pieterse, E., & van der Vlag, J. (2014). Breaking immunological tolerance in systemic lupus erythematosus. Frontiers in immunology, 5, 164.
13. Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D. S., ... & Zychlinsky, A. (2004). Neutrophil extracellular traps kill bacteria. science, 303(5663), 1532-1535.
14. Fuchs, T. A., Abed, U., Goosmann, C., Hurwitz, R., Schulze, I., Wahn, V., ... & Zychlinsky, A. (2007). Novel cell death program leads to neutrophil extracellular traps. The Journal of cell biology, 176(2), 231-241.
15. Hirai, Y., Iyoda, M., Shibata, T., Ashikaga, E., Hosaka, N., Suzuki, H., ... & Akizawa, T. (2008). Lupus nephritis associated with positive MPO-ANCA in a patient with underlying autoimmune hemolytic anemia. Clinical and Experimental Nephrology, 12(5), 393-397.
16. Said, D., Rashad, N. M., Abdelrahmanc, N. S., & Dawaa, G. A. (2021). Antineutrophil cytoplasmic antibody in lupus nephritis: correlation with clinicopathological characteristics and disease activity. Current Rheumatology Reviews, 17(2), 213-221.
17. Morgan, P. E., Sturgess, A. D., & Davies, M. J. (2005). Increased levels of serum protein oxidation and correlation with disease activity in systemic lupus erythematosus. Arthritis & Rheumatism, 52(7), 2069-2079.
18. Olson, S. W., Lee, J. J., Poirier, M., Little, D. J., Prince, L. K., Baker, T. P., ... & Abbott, K. C. (2017). Anti-myeloperoxidase antibodies associate

with future proliferative lupus nephritis. Autoimmune diseases, 2017.

19. Li, C., Wang, J. J., Zhou, M. L., Liang, D. D., Yang, J., Zhu, H. X., ... & Zhang, H. T. (2019). Differences in clinico-pathological characteristics and outcomes between proteinase 3-ANCA positivity and myeloperoxidase-ANCA positivity in lupus nephritis. Lupus, 28(9), 1111-1119.
20. Yu, F., Tan, Y., Liu, G., Wang, S. X., Zou, W. Z., & Zhao, M. H. (2009). Clinicopathological characteristics and outcomes of patients with crescentic lupus nephritis. Kidney international, 76(3), 307-317.