Glomerular Macrophage is an Indicator of Early Treatment Response in Diffuse Proliferative Lupus Nephritis

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Background: *Patients with diffuse proliferative lupus nephritis (class IV) who responded to treatment within 6 months had better renal outcome than those who did not. Glomerular macrophage is known to be associated with poor renal outcome in glomerular diseases.*
Objective: *To evaluate association between glomerular macrophage number and early treatment response in lupus nephritis class IV patients.*
Material and Method: *Renal biopsies (n = 90, 86 females) diagnosed with lupus nephritis class IV were included in the study. The patients were divided into 2 groups (n = 45 each) according to response to treatment within 6 months. The treatment response group was defined as having decreased serum creatinine at least 25% from baseline and 24 hr urine protein or UPCR (urine protein creatinine ratio) < 1. The non-response group was defined as stable or increased serum creatinine and 24 hr urine protein or UPCR ≥ 1. Immunohistochemistry for macrophage marker (CD68) was performed and the glomerular macrophages were counted on each biopsy. The relevant clinicopathologic data were collected.*
Results: *The glomerular macrophage number in response and non-response group was 4.5 ± 2.5 and 6.2 ± 4.5 respectively (p = 0.029). The glomerular macrophage number was conversely and inversely correlated with activity (r = 0.281, p = 0.007) and chronicity (r = -0.358, p < 0.001) index, respectively.*
Conclusion: *Lupus nephritis class IV patients who responded to treatment within 6 months had lower glomerular macrophages than those who did not. The glomerular macrophage number may be used to determine treatment response in lupus nephritis class IV patients.*

Keywords: *Lupus nephritis, Macrophages, Immunohistochemistry, Treatment response, Prognostic factor*

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Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease affecting multiple organs. The renal involvement poses the greatest risk for morbidity and mortality. Renal manifestation of SLE or lupus nephritis is found in 60% of patients at any time in the course of disease and most patients develop lupus nephritis early in the disease process[1].

A number of clinicopathologic parameters associate with renal outcome including proteinuria, serum creatinine and pathologic classification. Patients who had early treatment response had better renal outcome than those who did not[2]. Although parameters such as race, pathologic classification and chronicity index have been associated with outcome[3], there is currently no information regarding which clinicopathologic parameters are associated with this early treatment response. These parameters can be helpful in predicting renal outcome and patient management.

Glomerular macrophages had been associated with parameters indicating poor outcome such as serum creatinine, proteinuria and doubling serum creatinine time[4,5]. They had been shown to be involved in pathogenesis of proliferative glomerulonephritis[6,7] .

The primary objective of our study is to evaluate the association between glomerular

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macrophage number and treatment response at 6 months in diffuse proliferative lupus nephritis (class IV) patients. The secondary objective is to find association between glomerular macrophage number and relevant clinicopathologic parameters.

Material and Method

Patients and renal biopsies

Renal biopsies from 90 patients with lupus nephritis class IV retrieved from archive at Department of Pathology, Siriraj Hospital, Mahidol University from January 2005 to February 2008 were selected for study (see the statistical analysis section for the sample size). The study was approved by the Institutional Review Board at Faculty of Medicine Siriraj Hospital, Mahidol University. All the biopsies (n = 90, Male = 4, Female = 86) are diagnosed consecutively with lupus nephritis class IV according to the 2005 International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification[8]. The patients were not treated with cytotoxic drugs or corticosteroid before biopsies. Excluded from the study were patients who had less than 6 months of time after biopsies till the last follow-up and patients whose biopsies had less than 10 glomeruli in a specimen for light microscopy. The patients were divided into 2 groups according to response to treatment within 6 months (n = 45 each). The treatment response group was defined as having decreased serum creatinine at least 25% from baseline and 24 hr urine protein or UPCR (urine protein creatinine ratio) < 1. The non-response group was defined as stable or increased serum creatinine and 24 hr urine protein or UPCR (urine protein creatinine ratio) $\geq$ 1. The clinicopathologic data were collected including demography, blood pressure, treatment regimen, serum creatinine, UPCR, 24 hr urine protein at the biopsy time and 6 months after treatment, histologic activity and chronicity indices. The laboratory data obtained within 2 weeks before or after biopsy were included for analysis.

Immunohistochemistry

Immunohistochemistry was performed on formalin-fixed paraffin-embedded tissues following a standard avidin-biotin complex (ABC) method. Briefly, the renal biopsies were deparaffinized in xylene and rehydrated in graded ethanol. Endogenous peroxidase was blocked by incubation in 3% hydrogen peroxide. The sections were then incubated for one night with antibody to the macrophage marker CD68 (Diagnostic Biosystems, Pleasanton, California, USA) diluted in phosphate-buffered saline (PBS) plus 1% bovine serum albumin (BSA) at the dilution of 1:100. After washing in PBS, the sections were sequentially incubated with secondary antibody for 30 minutes, and finally 3,3′-diaminobenzidine (DAB; with nickel chloride enhancement) was used as the chromogen. Sections were counterstained with hematoxylin and were dehydrated and cover-slipped.

Glomerular macrophages number

Glomerular macrophages identified by CD68 staining were counted in each biopsy. The average number of macrophage per one glomerulus was calculated per one patient and then for the response and non-response group. The average number of glomerular macrophage in each group was used for analysis. All glomeruli except those showing global sclerosis were evaluated. Glomerular macrophage count was independently performed by 2 pathologists who were blinded to the treatment response. The slides were reviewed together by both pathologists if the results were significantly different.

Statistical analyses

A pilot study in 10 patients from each response and non-response group showed that the number of macrophage/glomerulus had a mean $\pm$ SD of 4.4 $\pm$ 2.1 and 7.1 $\pm$ 5.8 respectively. A sample size of 42 patients per group was calculated. However, 45 patients were selected in each group due to availability of cases during the study period.

Statistical analyses were performed using SPSS program version 18. Macrophage number/glomerulus in response and non-response group along with clinical and pathological data were compared by Chi-square test for categorical data and Student t-test for continuous data. The correlation between the glomerular macrophage number and serum creatinine, 24 hour urine protein, UPCR, activity and chronicity indices was demonstrated by Spearman's rank correlation.

Results

Clinical and pathological data comparing patients in response and non-response group are demonstrated in Table 1. Urine protein 24 hr and UPCR which are the defining features of early treatment response, are significantly lower in response group.

Mean glomerular macrophage number

A representative figure of lupus nephritis

class IV is given in Fig. 1A. Fig. 1B shows macrophages identified by immunohistochemical staining for CD68. The glomerular macrophage number was 4.5 ± 2.5 in response group and 6.2 ± 4.5 for non-response group. There was significant difference in glomerular macrophage number between the two groups, p = 0.029 (Table 1).

The number of glomerular macrophage was correlated conversely with the activity index and inversely with chronicity index. However, there was no correlation between glomerular macrophage number and other clinical data (Table 2).

Discussion

The present study demonstrate that glomerular macrophage number in the patients with lupus nephritis class IV who responded to the standard immunosuppressive treatment within 6 months is significantly lower than in patient who did not respond. In lupus nephritis, the number of glomerular macrophage infiltration had been shown to correlate with serum creatinine, proteinuria and progression to end stage renal disease[4,5].

Macrophages play crucial role in inflammatory process. They have been identified in glomeruli in most of inflammatory glomerular diseases[9]. In addition to primary injury caused by macrophages in glomeruli, the recruiting of macrophages into tubulointerstitial compartment also results in tubular cell injury[10]. In lupus nephritis they have been recruited to the inflamed glomeruli by the chemotactic factors especially monocyte chemoattractant protein 1 (MCP-1)[11] after immune complex deposition and complement activation[12]. Their activation either by interferon γ together with proinflammatory cytokines such as lipopolysaccharide (LPS), tumor necrosis factor α (TNF-α) or direct engagement of toll-like receptor leads to a proinflammatory phenotype[13,14]. These activated macrophages also produce pro-inflammatory cytokines, radical oxygen species and enzymes resulting in tissue injury[15,16]. Number of glomerular macrophages had been correlated with progression of glomerulonephritis in animal models and the reduction in their number resulted in decreased proteinuria and increased renal function[17-20].

The reason why lupus nephritis patients with lower glomerular macrophage number in the present study were more likely to respond to treatment than

Table 1. Comparison of clinical and pathological data between response and non-response patients

Clinical and pathological data		Response (n = 45)	Non-response (n = 45)	p
Age (year)		28.9 ± 11	30.6 ± 9.6	0.444
Gender (% Female) (n = 90)		43 (95.6%)	43 (95.6%)	1.000
Mean blood pressure (mmHg)	Systolic	147 ± 19.5	141 ± 24.0	0.235
	Diastolic	89 ± 15.3	88 ± 17.9	0.777
Treatment (n = 84)	High dose	23 (57.5%)	20 (45.4%)	0.527
	Low dose	14 (35.0%)	19 (43.2%)	
	Others	3 (7.5%)	5 (11.4%)	
SCr0 (mg/dL)		1.4 ± 0.8	1.3 ± 0.9	0.701
UPCR0		4.2 ± 2.6	6.6 ± 5.1	0.11
U24hr0 (g/day)		4.1 ± 2.5	6.5 ± 4.2	0.03
SCr6 (mg/dL)		0.9 ± 0.3	1.1 ± 0.9	0.189
UPCR6		0.5 ± 0.3	3.2 ± 2.2	< 0.001
U24hr6 (g/day)		0.6 ± 0.3	4.2 ± 2.5	< 0.001
AI		9.0 ± 2.3	9.2 ± 2.9	0.753
CI		3.5 ± 2.0	3.9 ± 2.4	0.373
No of MO/glomerulus		4.5 ± 2.5	6.2 ± 4.5	0.029

All values are mean ± SD except where noted. SCr0 = serum creatinine at biopsy time, UPCR0 = urine protein creatinine ratio at biopsy time, U24hr0 = 24 hr urine protein at biopsy time, SCr6 = serum creatinine at 6 months after treatment, UPCR6 = urine protein creatinine ratio at 6 months after treatment, U24hr6 = 24 hr urine protein at 6 month after treatment, MO = macrophage, AI = activity index, CI = chronicity index, High dose = intravenous cyclophosphamide regimen (6 monthly pulses and 2 quarterly pulse with escalating dose), Low dose = low dose intravenous cyclophosphamide (6 pulses of 500 mg at interval of 2 weeks), Others = azathioprine, mycophenolic acid, oral cyclophosphamide, rituximab, mycophenolate mofetil; $p < 0.05$ is considered statistically significant

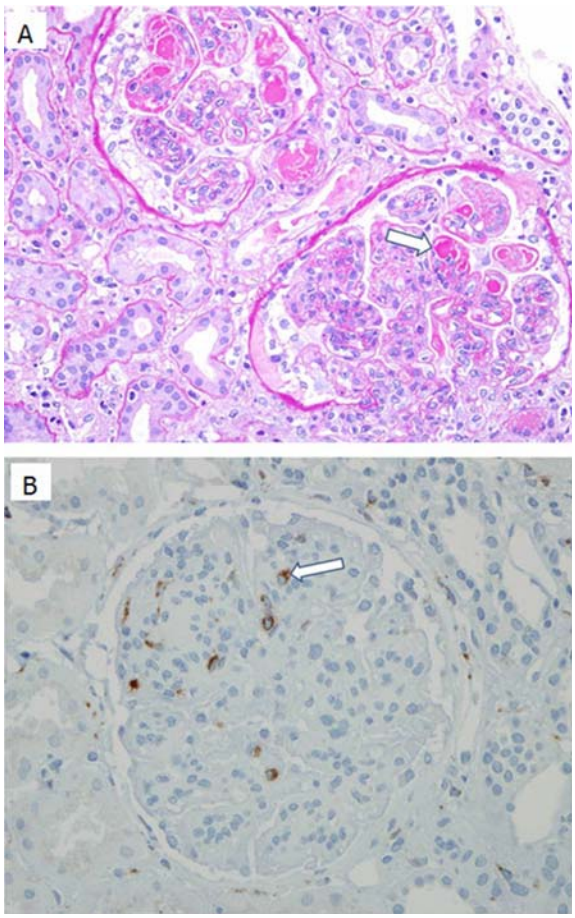

Fig. 1 Diffuse proliferative lupus nephritis (class IV) demonstrating mesangial cell proliferation, increased infiltration of neutrophils, mononuclear cells and macrophages, and depositions of immune complex (arrow). Extracapillary proliferation may be found in severe inflammatory process (Fig. 1A. Hematoxylin & Eosin, X400). Intraglomerular CD68 immunoperoxidase staining indicating glomerular macrophage infiltration (Fig. 1B, X400)

patients who had more macrophages may be due to more irreversible injury caused by macrophages in the latter group. There are a number of mechanisms in which macrophages can cause irreversible injury to the kidney. Activated macrophages can induce apoptosis of mesangial cells by producing nitric oxide[21]. Moreover, they can induce myofibroblast proliferation and extracellular matrix production[17,22]. These myofibroblasts can be found in glomerular crescentic lesion which is common in lupus nephritis class IV and in mesangium. The laying down of an excessive amount of extracellular matrix in glomeruli leads to glomerulosclerosis or glomerular scarring. In addition to irreversible glomerular injury, the spilling of glomerular macrophages into tubules and interstitium also induces tubular atrophy and interstitial fibrosis[10], the most important factors determining kidney survival in any glomerular, tubulointerstitial or renal vascular diseases[23].

Table 2. Correlation between clinicopathologic data and glomerular macrophage number

Clinicopathological data	Glomerular macrophage number	
	r	p
SBP0	0.003	0.979
DBP0	0.031	0.779
SCr0 (mg/dl)	-0.181	0.088
SCr6 (mg/dl)	-0.193	0.069
UPCR0	-0.084	0.458
UPCR6	0.001	0.994
U24hr0 (g/day)	-0.169	0.124
U24hr6 (g/day)	-0.079	0.621
AI	0.281	0.007
CI	-0.358	< 0.001

SBP0 = Systolic blood pressure at biopsy time, DBP0 = Diastolic blood pressure at biopsy time, Cr0 = Serum creatinine at biopsy time, Cr6 = Serum creatinine at 6 month after biopsy UPCR0 = Urine protein creatine ratio at biopsy time, UPCR6 = Urine protein creatine ratio at 6 month after biopsy, U24hr0 = 24 hour urine protein at biopsy time, U24hr6 = 24 hour urine protein at 6 month, AI = activity index, CI = chronicity index, $p < 0.05$ is considered statistically significance

There is correlation between proteinuria and glomerular macrophage number in a previous study[4]. However, no correlation is established in the present study. The reason is possibly due to the inclusion of cases with the features of membranous lupus nephritis or class V in the previous study. This feature is associated with heavy proteinuria in the nephrotic range. The effect of macrophages on proteinuria in that study might be different from the present study in which only cases with pure class IV were included.

Although there is statistical difference in number of glomerular macrophages between patients who responded and who did not respond to treatment, the cut off number cannot be calculated due to small sample size. In order to use glomerular macrophage number as a predictor for treatment response in clinical practice, a single value as cut off number should be derived. This task could be accomplished in a future follow-up prospective study with a larger sample size.

Conclusion

The present study has shown that diffuse proliferative lupus nephritis (class IV) patients who responded to treatment within 6 months had lower glomerular macrophages than those who did not. Counting glomerular macrophage number may be useful in determining early response to treatment in patients with lupus nephritis class IV.

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Potential conflicts of interest

None.

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การใช้จำนวนแมคโครฟาจในโกลเมอรูลัสเป็นตัวบ่งชี้การตอบสนองต่อการรักษาในระยะเริ่มต้นของผู้ป่วยโรคไตลูปัสแบบ diffuse proliferative

บุณยฤทธิ์ ชื่นสุชน, พิมพิณ อินเจริญ, รัตนา ชวนะสุนทรพจน์, ทวี ชาญชัยรุจิรา, ชัยรัตน์ ฉายากุล

วัตถุประสงค์*: เพื่อศึกษาความสัมพันธ์ระหว่างจำนวนแมคโครฟาจในโกลเมอรูลัส กับการตอบสนองต่อการรักษาในระยะ 6 เดือนแรก ในผู้ป่วยโรคไตลูปัส class IV*

วัสดุและวิธีการ*: ได้ทำการศึกษาในผู้ป่วยโรคไตลูปัส class IV (N = 90, หญิง 86 ราย) ที่ได้รับการวินิจฉัยโดยการตรวจทางพยาธิวิทยาของชิ้นเนื้อไต ผู้ป่วยถูกจำแนกเป็น 2 กลุ่ม โดยมีจำนวนกลุ่มละ 45 คน ตามการตอบสนองต่อการรักษาภายในระยะเวลา 6 เดือน ผู้ป่วยกลุ่มที่ตอบสนองต่อการรักษาจะมีปริมาณ serum creatinine และโปรตีนในปัสสาวะ 24 ชั่วโมงลดลง หรือค่า UPCR (urine protein creatinine ratio) < 1 ผู้ป่วยกลุ่มที่ไม่ตอบสนองต่อการรักษามีระดับ serum creatinine ที่คงที่หรือเพิ่มขึ้น และโปรตีนในปัสสาวะ 24 ชั่วโมง หรือ UPCR ≥1 ชิ้นเนื้อไตที่ได้จากผู้ป่วยถูกนำไปย้อมด้วยวิธี immunohistochemistry ด้วย antibody ที่จำเพาะต่อแมคโครฟาจ (CD68) จากนั้นจึงนับจำนวนแมคโครฟาจในโกลเมอรูลัสในชิ้นเนื้อไตที่ได้จากผู้ป่วยทุกราย นอกจากนี้ได้รวบรวมข้อมูลทางคลินิกและทางพยาธิวิทยาอย่างอื่นที่เกี่ยวข้องกับโรคเพื่อศึกษาเปรียบเทียบระหว่างทั้งสองกลุ่มนี้ด้วย*

ผลการศึกษา*: จำนวนแมคโครฟาจในโกลเมอรูลัสในกลุ่มผู้ป่วยที่ตอบสนองต่อการรักษาเท่ากับ 4.5 ± 2.5 ในกลุ่มที่ไม่ตอบสนองเท่ากับ 6.2 ± 4.5 (p = 0.029) นอกจากนี้ยังพบว่าจำนวนแมคโครฟาจที่ลดลงสัมพันธ์กับ chronicity index ที่เพิ่มขึ้น (r = -0.369, p < 0.001) และจำนวนที่เพิ่มขึ้นสัมพันธ์กับ activity ที่เพิ่มขึ้น (r = 0.281, p = 0.007)*

สรุป*: ผู้ป่วยโรคไตลูปัส class IV ที่ตอบสนองต่อการรักษาภายใน 6 เดือนมีจำนวนแมคโครฟาจในโกลเมอรูลัสในขณะเริ่มต้นการรักษาน้อยกว่าในผู้ป่วยที่ไม่ตอบสนอง จำนวนแมคโครฟาจในโกลเมอรูลัสอาจเป็นตัวชี้วัดอันหนึ่งในการบอกว่าผู้ป่วยโรคไตลูปัส class IV จะตอบสนองต่อการรักษาด้วยยากดภูมิภายในระยะเวลา 6 เดือนแรก*