

Autophagy - related protein 5: A promising biomarker for disease activity and treatment response in lupus nephritis

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Abstract

Existing biomarkers are often insufficient for adequately tracking treatment outcomes in lupus nephritis (LN). Growing evidence points to a significant role of autophagy in LN pathogenesis. This study aimed to evaluate circulating levels of autophagy-related protein 5 (ATG5) alongside its immunohistochemical expression in kidney biopsy from LN patients, and to examine its utility as a diagnostic indicator and predictor of therapeutic response. This pilot prospective research enrolled 31 patients with active LN and 9 age and sex matched normal controls. Immunohistochemical evaluation of ATG5 expression was conducted on renal biopsy specimens collected before the commencement of medication, while blood ATG5 levels were evaluated in all subjects. Serial serum ATG5 evaluations were performed at 3-month and 6-month intervals after the initiation of therapy. Circulating ATG5 levels were significantly decreased in LN patients relative to controls (cut-off ≤ 312.3 ng/l; AUC=0.943; sensitivity 100%; specificity 88.9%). Renal tissue ATG5 expression was detected in 96.7% of patients; strong expression identified in 87.1% using summation scoring (score >3) and in 58.1% using multiplication scoring (score >8). A biopsy summation score ≤ 6 predicted treatment response with AUC of 0.88, sensitivity 69.57% and 100% specificity. Treatment was associated with significant ATG5 recovery over 6 months ($p < 0.001$), with responders demonstrating lower biopsy scores and greater serum ATG5 improvement versus non-responders ($p = 0.004$, $p = 0.009$, respectively). Mycophenolate mofetil (MMF) treatment yielded superior outcomes compared to cyclophosphamide ($p = 0.042$). The summation and multiplication scores of ATG5 exhibited a negative correlation with serum ATG5 after six months ($p < 0.026$ and $p < 0.018$, respectively). In conclusion, ATG5 represents a valuable autophagy-related biomarker in LN, offering reliable predictive utility for treatment outcomes. Lower intra-renal ATG5 expression scores are associated with favourable therapeutic responses, particularly in patients receiving MMF.

Keywords: Autophagy; ATG5; Lupus nephritis, eGFR.

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Introduction

Systemic lupus erythematosus (SLE) is a long-standing autoimmune disorder, characterized by loss of immunological self-tolerance, resulting in polyclonal autoantibody production, widespread immune complex deposition in various organs, and persistent inflammation with consequent tissue injury.¹ Among its most serious manifestations, lupus nephritis (LN) develops in roughly half of all SLE patients, typically within five years of initial diagnosis, with a subset progressing to permanent renal impairment.² Despite decades of research, the underlying mechanisms driving LN remain only partially elucidated.

Standard treatment relies heavily on corticosteroids and immunosuppressive agents, yet therapeutic responses are frequently incomplete, and long-term use carries considerable toxicity.³ These limitations underscore the urgent need for more precise diagnostic biomarkers and targeted therapeutic strategies.³ The highly conserved intracellular degradation process, known as autophagy transports cytoplasmic components to lysosomes for recycling and disintegration.⁴ Beyond its essential function in preserving cellular homeostasis, autophagy was linked to the pathophysiology of numerous disease states, including cancers, infections, autoimmune diseases, neurological and cardiovascular diseases.⁵ Autophagy has a fundamental role in regulating T-cell and B-cell survival and function.⁶ Genome-wide association studies have identified multiple susceptibility loci for SLE, some of which are functionally linked to immune regulation.⁷

Multiple cell types, including B cells, T cells, dendritic cells, and phagocytes, are involved in the dysregulation of both innate and adaptive immunity in SLE.⁸ According to experimental findings; autophagy plays a part in this process. For instance, *Clarke et al.*, 2015⁹ found that splenic CD19⁺ B cells from NZB/W lupus prone mice had considerably more autophagic activity than controls, and comparable changes were seen in B cells from SLE patients. Furthermore, *Conway et al.*, 2013¹⁰ showed that late-stage B-

cell activation and differentiation into plasma cells depend on the autophagy 5 (ATG5) related protein. Given that LN is driven by immune-mediated mechanisms, dysregulated autophagy may contribute to both systemic autoimmunity and renal injury.⁹ Accordingly, assessment of autophagy-related markers could have potential clinical relevance. In this context, the present study evaluated serum ATG5 levels and its expression in renal biopsy specimens from patients with LN, and examined their association with disease severity, histopathological findings, and response to therapy.

Patients and Methods

This prospective pilot research study was performed in the Nephrology units of Ain Shams University Hospitals and included 31 adult patients with biopsy-confirmed active proliferative LN (Class III or IV). The eligibility criteria were proteinuria above 0.5 g/day, active urinary sediment, and increased serum creatinine levels. A control group of nine age- and sex-matched normal individuals was also recruited for comparison of serum ATG5 levels.

Patients were excluded if they had end-stage renal disease, non-proliferative LN, or other causes of renal impairment, including diabetic nephropathy and obstructive uropathy. Additional exclusion criteria included heart failure, active infection or sepsis, history of nephrolithiasis, pregnancy-associated lupus nephritis, anti-phospholipid syndrome-related nephropathy, and critical illness requiring intensive care admission.

All subjects had comprehensive clinical assessments, including an extensive medical history and physical examination. All the following data were collected from the hospital records. Laboratory data included complete blood count (CBC), blood urea nitrogen, serum creatinine, serum albumin, serum electrolytes (sodium, potassium, calcium, and phosphate), uric acid, urinalysis, urine protein-to-creatinine ratio, C-reactive protein (CRP), complement fractions (C3 and C4), antinuclear antibodies

(ANA), anti-double-stranded DNA (anti-dsDNA) antibody titres, and serum ATG5 concentration.

Renal biopsy specimens were collected and evaluated for ATG5 expression before initiation of treatment. Treatment outcomes were stratified into three groups. A complete response was defined by a normal estimated glomerular filtration rate (eGFR) or a reduction not exceeding 25% from baseline Modification of Diet in Renal Disease (MDRD)-GFR, together with proteinuria <0.5 g/day. A partial response was characterized by similarly preserved eGFR and at least a 50% decrease in proteinuria, with remaining levels between 0.5 g and 3.0 g/day. Patients not meeting either definition were considered non-responders.

Pathological assessment of the renal biopsy with ATG5 protein

All patients had renal biopsy at the time of active LN diagnosis as a component of standard clinical practice. Biopsy specimens were treated using normal histopathological methodologies, including light microscopy, immunofluorescence or immunohistochemical staining for IgG, IgM, IgA, and C3, as well as electron microscopy. Light microscopic observations were used to categorize LN based on the modified International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification system, while disease activity and chronicity were assessed using the National Institutes of Health (NIH) activity and chronicity indices, respectively.

ATG5 expression in renal tissue was assessed using immunohistochemistry staining, conducted before the commencement of therapy. Formalin-fixed, paraffin-embedded tissue slices, each 4 µm thick, were produced from all 31 biopsy specimens in accordance with normal deparaffinization and rehydration protocols. Staining was performed with an automated immunostainer (BenchMark Ventana GX) using Cell Conditioning 1 (CC1) standard antigen retrieval pre-treatment. Sections were incubated overnight at 4°C with anti-ATG5 primary antibody (Catalog No. A0203, ABclonal Germany GmbH), followed by the application of the VENTANA ultra View

Universal DAB Detection Kit (Catalog No. 760-500; Ventana Medical Systems, Inc., Tucson, AZ, Roche Diagnostics; USA) according to the manufacturer's instructions. Following the staining procedure, slides were washed, dehydrated with graded alcohol, cleaned with xylene, and mounted with Canada balsam under a coverslip. To ensure impartiality, all immunohistochemistry findings were assessed separately by pathologists who were unaware of the clinical and pathological information pertaining to each patient.

ATG5 expression was assessed using two complementary scoring methods for each biopsy specimen. Both methods incorporated three parameters: staining intensity (0 = negative, 1 = weak, 2 = moderate, 3 = strong), fraction of positive cells (0 = negative, 1 = 1–24%, 2 = 25–75%, 3 = >75%), and distribution pattern (0 = negative, 1 = focal, 2 = diffuse). In the multiplication scoring method, the three parameters were multiplied to generate a composite score ranging from 0 to 27. In the summation scoring method, the parameters were added to obtain a total score ranging from 0 to 8. Representative staining patterns are illustrated in Figures 1 and 2.

Laboratory assessment of Serum ATG5

A venous blood sample (5 ml) was drawn from each participant and allowed to clot for 10 minutes at room temperature (18–25°C). Samples were then centrifuged at 1500 × g for 20 minutes, and the serum was carefully separated. Samples exhibiting visible precipitates were subjected to a second centrifugation before analysis. Serum ATG5 levels were measured using a double-antibody sandwich enzyme-linked immunosorbent assay with a monoclonal antibody specific for human ATG5 (Catalog No. 201-12-5694, Shanghai Sunred Biological Technology Co., Ltd., Shanghai, China).

Statistical Analysis

All data were coded, tabulated, and analysed using the Statistical Package for the Social Sciences (SPSS) IBM (version 28). Categorical data were analysed using the chi-square test. Continuous variables were compared between

groups with the independent samples t-test when normally distributed, and with the Mann-Whitney U test for non-normally distributed data. For comparisons involving three groups, one-way analysis of variance (ANOVA) was applied. Associations between variables were evaluated using the Pearson's correlation coefficient for parametric data and Spearman's

rank correlation for non-parametric data. Post hoc pairwise analyses were conducted with Bonferroni adjustment. The receiver operating characteristic (ROC) curve analysis was performed to determine the optimum diagnostic ability of a binary classifier. A $p \leq 0.05$ was considered statistically significant.

<u>Score description of ATG5 glomerular staining</u>		
<u>> INTENSITY</u>	<u>> Ratio of positive cells</u>	<u>> Pattern of Distribution</u>
• 0 = Negative staining	• 0 = Negative	• 0 = Negative
• 1 = Weak staining	• 1 = 1-24%	• 1 = Focal
• 2 = Moderate staining	• 2 = 25-73%	• 2 = Diffuse
• 3 = Strong staining	• 3 = > 75%	
<u>> A score > 8 was described as strong expression for multiplication and > 3 for summation.</u>		

Figure 1. Autophagy-related protein 5 (ATG5) staining summation and multiplication score in renal biopsies in lupus nephritis (LN) patients.



Figure 2. Expression of autophagy-related protein 5 (ATG5) in renal biopsy of lupus nephritis (LN).

Results

The demographic and clinical features of all research participants are detailed in Table 1. ATG5 positivity in renal biopsy specimens was detected in 87.1% of patients (n=27) based on a

summation score exceeding 3, and in 58.1% (n=18) when a multiplication score greater than 8 was applied as the threshold.

Table 1. Demographic data of the 31 patients included in the study and renal biopsy findings.

Patients group (n= 31)	
Age (Years) Mean $\pm$ SD	32.48 $\pm$ 11.18
Sex	
Female	20 (64.5%)
Male	11 (35.5%)
Class	
III	12 (38.7%)
IV	19 (61.3%)
Response	
Non responder	8 (25.8%)
Partial responder	18 (58.1%)
Complete responder	5 (16.1%)
Protocols of treatment	
MMF	11 (35.5%)
Cyclophosphamide	20 (64.5%)
ATG5 Biopsy multiplied score /18	
Median (IQR)	12(6 – 12)
≤ 8	13 (41.9%)
> 8	18 (58.1%)
ATG5 Biopsy score by Summation /8	
Median (IQR)	6 (5 – 7)
≤ 3	4 (12.9%)
> 3	27 (87.1%)

Baseline serum ATG5 concentrations were markedly lower in LN patients prior to initiation of treatment (230.59 ± 3.35 ng/l) than in normal controls (1049.94 ± 330.83 ng/l), ($p < 0.001$), as shown in Table 2. The ROC curve analysis identified an optimal diagnostic cut-off of

≤ 312.3 ng/l, yielding an area under the curve (AUC) of 0.943, with a sensitivity of 100%, specificity of 88.9%, positive predictive value (PPV) of 96.9%, and negative predictive value (NPV) of 100% (Figure 3).

Table 2. Comparison of age, sex and serum ATG5 (baseline) between the control group and patients' group.

		Control groups	Patients' groups	p-value
		No. = 9	No. = 31	
Age (years)	Mean $\pm$ SD	29.44 $\pm$ 6.06	32.48 $\pm$ 11.18	NS•
	Range	19 – 39	18 – 57	
Sex	Female	4 (44.4%)	20 (64.5%)	NS*
	Male	5 (55.6%)	11 (35.5%)	
ATG5 (ng/L) (baseline)	Mean $\pm$ SD	1049.94 $\pm$ 330.83	230.59 $\pm$ 3.35	<0.001•
	Range	236.4 – 1200	114.4 – 312.3	

$p > 0.05$ is not significant (NS). •: Chi-square test. *: Independent t-test.

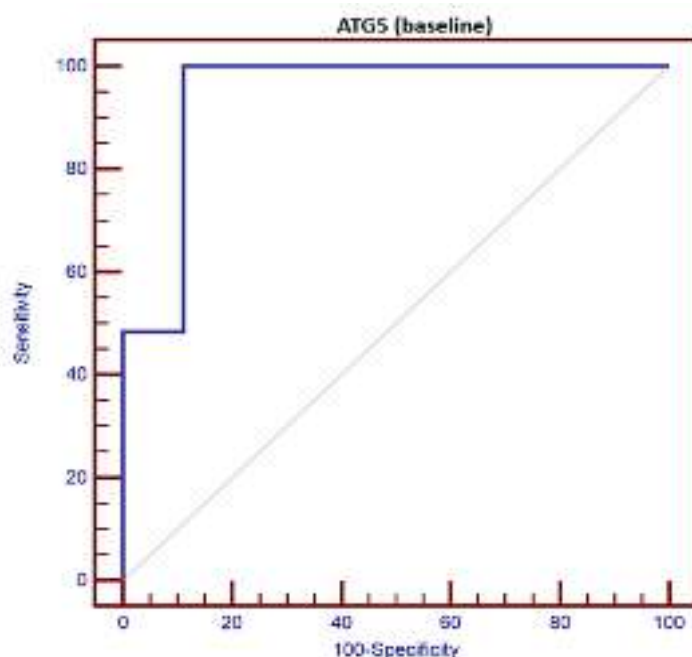


Figure 3. Receiver operating characteristic curve (ROC) for autophagy-related protein 5 (ATG5, baseline) in patients with active lupus nephritis.

Therapy with mycophenolate mofetil (MMF) yielded significantly better outcomes than cyclophosphamide ($p=0.042$). In the MMF group, 72.7% of patients achieved partial remission and 27.3% reached complete remission, whereas the corresponding rates in the cyclophosphamide group were 50% and 10%. Patients who attained either complete or partial remission exhibited significantly lower ATG5 summation and multiplication scores in renal biopsy samples, along with higher baseline serum ATG5 levels ($p=0.004$, $p=0.009$, and $p=0.022$, respectively), as shown in Table 3. Compared to responders, non-responding patients showed significantly elevated ATG5 multiplication and summation scores in renal biopsy specimens ($p=0.012$ and $p=0.006$, respectively). In contrast, no statistically

significant differences in either serum ATG5 levels or biopsy ATG5 scores were found when partial and complete responders were compared with one another ($p=0.100$ and $p=0.334$, respectively), suggesting that ATG5 parameters do not discriminate between these two response categories (Table 3). In comparison to responders, non-responding patients had significantly greater ATG5 multiplication and summation scores in renal biopsy specimens ($p=0.012$ and $p=0.006$, respectively). No statistically significant differences in serum ATG5 levels or biopsy ATG5 scores were observed when comparing partial and complete responders ($p=0.100$ and $p=0.334$, respectively), indicating that ATG5 parameters do not differentiate between these response categories (Table 3).

Table 3. Comparison of baseline laboratory data, activity markers and biopsy finding and Post hoc analysis between lupus nephritis patients, non-responder, partial responder, and complete responder with treatment.

		Response			<i>p</i> -value
		Non responder	Partial response	Complete response	
		No. = 8	No. = 18	No. = 5	
Protocols	MMF	0 (0.0%)	8 (44.4%)	3 (60.0%)	0.042*
	Cyclophosphamide	8 (100.0%)	10 (55.6%)	2 (40.0%)	
ATG5 Biopsy multiplication /18	Median (IQR)	15 (12 – 18)	10 (6 – 12)	4 (1 – 12)	0.009#
ATG5 Biopsy Summation /8	Median (IQR)	7.5 (7 – 8)	6 (5 – 7)	5 (5 – 6)	0.004#
ATG5 (ng/L)	Mean ± SD	200.46 ± 43.5	252.42 ± 43.67	200.22 ± 69.43	0.022•
P/C (g/dl)	Median (IQR)	4.7 (4.2 – 5.4)	5.45 (3.7 – 7.3)	4.6 (4 – 8)	NS#
C3 (mg/dl)	Mean ± SD	71.63 ± 10.32	64.09 ± 13.86	65.2 ± 11.39	NS•
C4 (mg/dl)	Median (IQR)	11.5 (8 – 16)	8.55 (7.4 – 12)	7 (6 – 9)	NS#
Anti-DNA	Positive	6 (75.0%)	13 (72.2%)	4 (80.0%)	NS*
BUN (mg/dl)	Median (IQR)	73.5 (60.5 – 107)	45 (34 – 61)	28 (17 – 28)	0.001#
Creatinine (mg/dl)	Median (IQR)	4.05 (3.6 – 6.8)	3 (1.9 – 3.8)	1.4 (1.2 – 1.6)	0.001#
eGFR (ml/min)	Median (IQR)	14 (9.5 – 17.5)	21 (17 – 30)	51 (48 – 57)	0.001#
Post Hoc analysis by LSD test					
Parameters	Non responder vs Partial response	Non responder vs Complete response	Partial response vs Complete response		
ATG5 biopsy multiplication score	0.007	0.012	0.334		
ATG5 biopsy Summation score	0.003	0.006	0.446		
ATG5 (ng/l)	0.012	1.000	0.100		

p > 0.05 is not significant (NS).; *: Chi-square test; •: One Way ANOVA test; #: Kruskal-Wallis test

Responders exhibited significantly enhanced restoration of blood ATG5 levels at both 3 and 6 months following initiation of treatment in comparison to non-responders (*p*=0.001). Conventional serological markers such as ANA, anti-dsDNA, C3, and C4 did not exhibit statistically significant differences across the three response groups, underscoring the

superior efficacy of ATG5 as a therapy response measure compared to traditional biomarkers (Table 4). The ROC curve analysis revealed that a renal biopsy ATG5 summation score of ≤6 could predict treatment response with good diagnostic accuracy, achieving an AUC of 0.88, sensitivity of 69.57%, specificity of 100%, PPV of 100%, and NPV of 53.3% (Figure 4).

Table 4. Comparison between non-responder, partial responder and complete responder lupus nephritis patients as regard baseline laboratory data, activity markers after 3 and 6 months of treatment.

Labs after 3 months		Response			p-value
		Non	Partial	Complete	
		responder	response	response	
		No. = 8	No. = 18	No. = 5	
P/C (g/dl)	Median (IQR)	3.95 (3.3 – 4.95)	3.2 (2.7 – 3.9)	2.4 (2.1 – 3)	0.024 $\neq$
	Range	3 – 6.4	1.9 – 9	1.6 – 3.7	
ATG5 (ng/l)	Mean $\pm$ SD	243.18 $\pm$ 51.86	383.82 $\pm$ 76.01	464.88 $\pm$ 68.81	0.000 $\bullet$
C3 (mg/dl)	Mean $\pm$ SD	82.25 $\pm$ 7.32	83.72 $\pm$ 10.32	82 $\pm$ 9.03	NS $\bullet$
C4 (mg/dl)	Median (IQR)	17 (13.5 – 23.5)	14 (12 – 17)	17 (9 – 18)	NS $\neq$
ANA	Positive	8 (100.0%)	12 (66.7%)	4 (80.0%)	NS*
Anti-DNA	Positive	3 (37.5%)	9 (50.0%)	4 (80.0%)	NS*
P/C (g/dl)	Median (IQR)	3.6 (3.05 – 4.15)	1.85 (1.4 – 2.3)	0.5 (0.4 – 0.5)	0.000 $\neq$
ATG5 (ng/l)	Mean $\pm$ SD	272.89 $\pm$ 52.79	503.41 $\pm$ 106.64	661.28 $\pm$ 92.1	0.000 $\bullet$
C3 (mg/dl)	Mean $\pm$ SD	91.13 $\pm$ 8.51	94.06 $\pm$ 8.65	91.4 $\pm$ 7.7	NS $\bullet$
C4 (mg/dl)	Median (IQR)	21 (17 – 25.5)	15.5 (12 – 18)	14 (12 – 21)	NS $\neq$
ANA	Positive	2 (25.0%)	6 (33.3%)	2 (40.0%)	NS*
Anti-DNA	Positive	3 (37.5%)	5 (27.8%)	3 (60.0%)	NS*

$p > 0.05$ is not significant (NS).; *: Chi-square test; $\bullet$: One Way ANOVA test; $\neq$: Kruskal-Wallis test

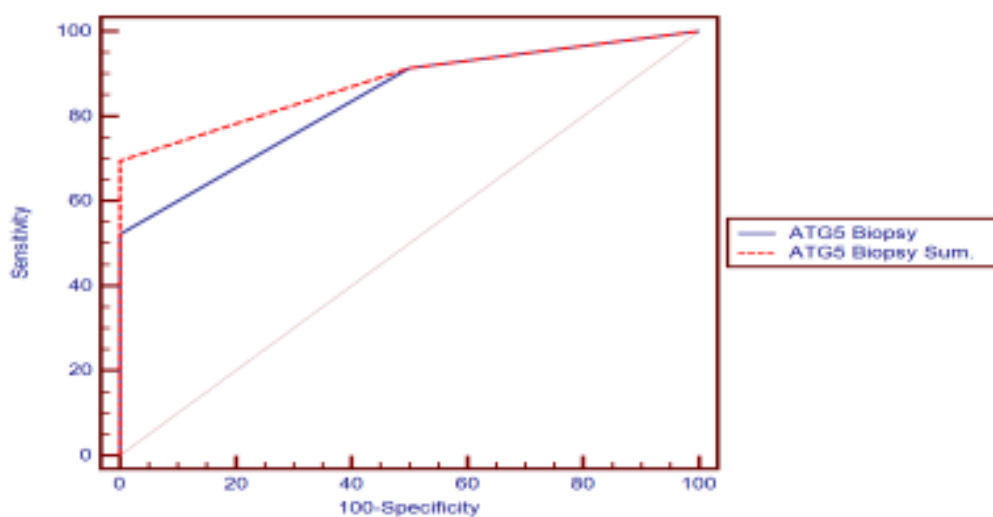


Figure 4. Receiver operating characteristic (ROC) curve for prediction of autophagy-related protein 5 (ATG5) responses in biopsy.

Post-treatment serum ATG5 concentrations at 3 and 6 months showed significant inverse correlations with both serum creatinine and urine protein-to-creatinine ratio, while demonstrating a significant positive correlation with eGFR ($p < 0.05$), as summarized in Table 5. Furthermore, ATG5 multiplication and

summation scores derived from renal biopsy specimens were both significantly and inversely correlated with serum ATG5 concentrations measured at 6 months ($r = -0.399$, $p = 0.026$ and $r = -0.421$, $p = 0.018$, respectively), the relationship is illustrated in Figure 5.

Table 5. Correlation between ATG5 at baseline, after 3 months and after 6 months with activity and laboratory parameters.

	ATG5 (Baseline)		ATG5 (3 months)		ATG5 (6 months)	
	r	p-value	r	p-value	r	p-value
ATG5 Biopsy multiplication	-0.195	NS	-0.292	NS	-0.399	0.026
ATG5 Biopsy Summation	-0.250	NS	-0.382	0.034	-0.421	0.018
Creatinine	-0.179	NS	-0.409	0.022	-0.488	0.005
C3	-0.048	NS	-0.212	NS	-0.246	NS
C4	0.083	NS	-0.163	NS	-0.218	NS
eGFR	0.124	NS	0.466	0.008	0.565	0.001

$p > 0.05$ is not significant (NS); r: Spearman correlation coefficient

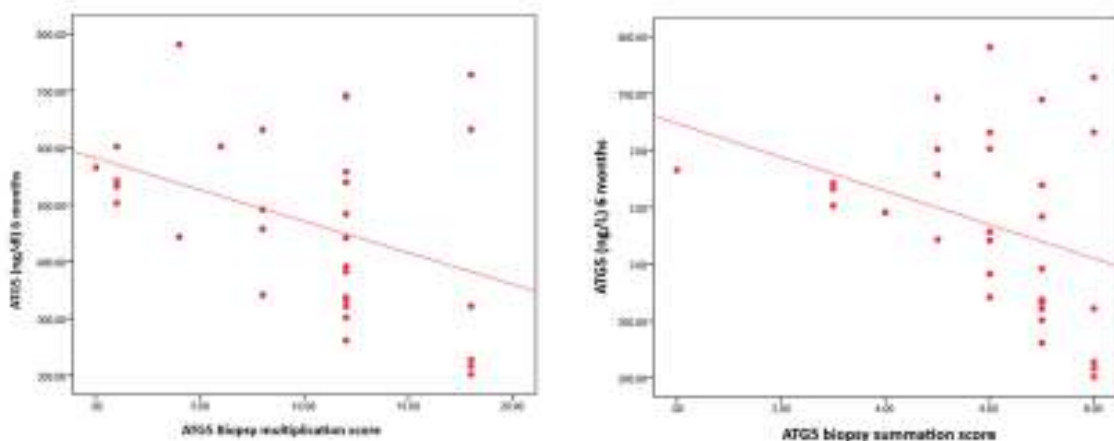


Figure 5. Correlation between autophagy-related protein 5 (ATG5) level after 6 months and ATG5 biopsy scores of the 31 studied patients.

Discussion

Effective biomarkers in LN should reflect renal-specific pathology rather than merely overall disease activity, as they are needed to predict renal flares and correlate with underlying histopathological changes.¹¹ Emerging evidence points to a mechanistic link between autophagy

dysregulation, SLE pathogenesis, and kidney injury, raising the possibility of autophagy-targeted therapeutic intervention.¹² From an immunological standpoint, ATG5 plays a pivotal role in autophagosome formation and is integral to antigen presentation, lymphocyte viability, and plasma cell maturation. Consequently, aberrant regulation of ATG5 may sustain T-cell

activation and promote auto-reactive B-cell responses, leading to persistent autoantibody production and immune complex-mediated damage.^{9,10,13} In this context, the present study investigated ATG5 expression in both serum and renal tissue of patients with proliferative LN. Our findings demonstrated that ATG5 is significantly dysregulated in active LN and holds potential as both a diagnostic and predictive biomarker, with key observations including markedly reduced circulating ATG5 levels, high prevalence of intra-renal expression, an inverse serum-tissue relationship, and strong associations with treatment response.

The demographic profile of our cohort was consistent with established patterns of LN presentation. The mean patient age was 32.48 ± 11.18 years, with a female predominance of 64.5% and a higher proportion of Class IV disease (61.3%) relative to Class III (38.7%). No significant differences in treatment response were identified based on sex, supporting prior evidence that histological class and baseline renal function are more reliable outcome predictors than sex alone (Table 3). These findings align with those of Lech and Anders., 2013,¹ who described LN as typically manifesting in the second to fourth decades of life with a marked female predominance, attributable to the influence of oestrogens on immune cell survival and function, particularly B cells and Plasmacytoid dendritic cells which serve as the major cellular producers of type I interferon, as well as the immunological contribution of X-linked genes.¹⁴ The study by Maroz and Segal., 2013² similarly reported that LN most commonly emerges within the first five years of SLE diagnosis in young women, further corroborating the demographic characteristics observed in our study population.

The predominance of Class IV LN in our cohort is consistent with patterns described in other proliferative LN series, where diffuse proliferative nephritis represents the most frequent and clinically severe histological subtype. The study by Jin et al., 2019¹⁵, examined the autophagy-related marker expression in LN renal tissue and observed a high proportion of Class IV cases in their study. The study by Almalki et al., 2019¹⁶, reporting on

a Saudi Arabian cohort of 96 proliferative LN patients, likewise documented a predominance of Class IV lesions with a comparable age distribution, indicating consistency in disease presentation across Middle Eastern populations that extends to our Egyptian cohort.

The current investigation revealed that serum ATG5 exhibited excellent diagnostic efficacy in differentiating LN patients from normal controls, with an optimum cut-off 312.3 ng/l, an AUC of 0.94, a sensitivity of 100%, and a specificity of 88.89% (Figure 3). The mean serum ATG5 level was significantly lower in patients with active LN compared to controls (230.59 ± 3.35 ng/l vs 1049.94 ± 330.83 ng/l; $p < 0.001$), indicating significant depletion of circulating ATG5 during episodes of renal inflammation (Table 2). These data align with those reported by the study of Alizadeh et al., 2018¹² and Zhou et al., 2011,¹⁷ who demonstrated a significant association between autophagy dysregulation and susceptibility to SLE. Genome-Wide Association Study revealed numerous genetic loci associated with susceptibility to SLE, and further investigations showed a specific link between polymorphisms in the positive regulatory domain I (PRDM1)–ATG5 intergenic region and increased disease risk.^{17,18} More recently, bioinformatics analyses identified autophagy-related genes including microtubule-associated protein 1 light chain 3 beta (MAP1LC3B) as diagnostically relevant features of LN renal tissue, correlating significantly with immune cell infiltration patterns.¹⁹ These results together underscore the critical role of autophagy dysregulation in systemic and renal immunological dysfunction in SLE. Taken together, these observations indicated that dysregulation of ATG5-associated autophagy is more likely to reflect active pathogenic processes in LN rather than representing a nonspecific inflammatory reaction. While previous investigations have largely focused on intracellular autophagic activity within immune cell populations⁹ and transcriptomic profiling of LN renal tissue,¹⁹ the present study extended these observations to the systemic compartment, proposing serum ATG5 as a potentially useful and minimally invasive biomarker pending further validation in

larger cohorts. The observed reduction in circulating ATG5 concentrations may reflect either enhanced tissue sequestration of the protein or disruption of normal autophagic flux in the setting of active renal inflammation, though the precise mechanism warrants further investigation.

Intra-renal ATG5 expression was seen in 96.7% of patients (n=30) with active proliferative LN, with robust expression noted in 87% by summation scoring (score >3) and in 58% using multiplication scoring (score >8). This finding indicated that ATG5 overexpression is an almost consistent feature of renal tissue in active proliferative LN. Experimental data indicated that podocyte autophagy is stimulated by inflammatory stress in LN, suggesting a dual function that concurrently promotes cell survival and may contribute to immune-mediated tissue damage.²⁰ Although these data provided biological credibility to the modified ATG5 tissue expression patterns seen in our cohort, the exact molecular connection between intra-renal ATG5 overexpression and reduced circulating levels has yet to be thoroughly elucidated.

Our findings align with those of Jin et al., 2019,¹⁵ who documented elevated autophagic activity in LN renal tissue, with strong ATG7 and Beclin-1 multiplication scores observed in 80% of Class III patients ($p=0.025$ and $p=0.009$, respectively) and 93.3% of Class IV patients ($p=0.002$ and $p<0.0001$, respectively) relative to normal controls. Notably, that study did not employ a summation scoring approach for biopsy evaluation. In the present study, we introduced the summation score as a complementary biopsy assessment tool, which demonstrated superior reflection of disease activity compared to multiplication scoring alone. Specifically, an ATG5 biopsy summation score of ≤ 6 predicted treatment response with an AUC of 0.88, sensitivity of 69.57%, and specificity of 100%, representing a meaningful methodological contribution to the field. From a mechanistic perspective, autophagy in stressed renal cells may serve a dual function, offering cytoprotection while simultaneously sustaining autoreactive lymphocyte survival, thereby creating a complex interplay between tissue

preservation and immune-mediated injury.^{12,21} In support of this notion, we observed a consistent inverse relationship between circulating ATG5 levels and intra-renal expression, suggesting a redistribution phenomenon whereby increased tissue sequestration of ATG5 drives reductions in serum concentrations. Although the observed correlations were of modest magnitude, their directional consistency supports a pathophysiological connection between intra-renal autophagic activation and systemic ATG5 dynamics.

Treatment response distribution in our cohort revealed that 8 patients (25.8%) were non-responders, 18 (58.1%) achieved partial response, and 5 (16.1%) attained complete response. Baseline serum ATG5 was significantly lower in non-responder patients compared to partial responders (200.46 ± 43.5 ng/l vs 252.42 ± 43.67), with post hoc analysis confirming this difference to be statistically significant (Table 3). Interestingly, complete responders showed comparable baseline serum ATG5 to non-responders (200.22 ± 69.43 ng/l), suggesting that baseline serum ATG5 alone may not fully discriminate between all response categories and should be interpreted alongside biopsy scoring data. Traditional serological biomarkers—namely antinuclear antibodies (ANA), anti-double-stranded DNA (anti-dsDNA), complement component 3 (C3), and complement component 4 (C4) exhibited limited and inconsistent associations with treatment outcomes, consistent with observations by Almaani et al., 2017²² who emphasized the inadequacy of standard serological markers in capturing renal-specific disease activity in LN.

MMF-treated patients achieved significantly better treatment outcomes than those receiving cyclophosphamide, consistent with findings reported by Almalki et al., 2019,¹⁶ who demonstrated superior remission rates with MMF as induction therapy in proliferative LN. Post-treatment serum ATG5 levels at both 3 and 6 months correlated inversely with serum creatinine and urine protein-to-creatinine ratio, and correlated positively with eGFR (Table 5), further supporting ATG5 as a reliable marker of

renal functional recovery. ATG5 biopsy summation and multiplication scores additionally showed significant inverse correlations with serum ATG5 at 6 months (Figure 5), reinforcing the serum-tissue redistribution relationship described above. From a mechanistic standpoint, MMF exerts its primary immunosuppressive effect through inhibition of inosine monophosphate dehydrogenase (IMPDH), thereby selectively restricting guanosine nucleotide synthesis in activated T and B lymphocytes.²³ Beyond this anti-proliferative action, MMF may additionally modulate intracellular autophagy-related signalling pathways, though the precise nature of this interaction has yet to be fully elucidated.^{23,24}

This study has several limitations that should be acknowledged. The modest sample size and the predominance of female participants may constrain the broader applicability of the results, although this distribution is consistent with the known female bias in SLE. In addition, evaluation of autophagy was confined to ATG5, and incorporating other markers such as ATG7 and Beclin-1 multiplication scores in future research would allow for a more thorough assessment of autophagic processes in LN. Moreover, only patients with proliferative forms of LN (Class III and IV) were included, leaving it unclear whether ATG5 expression varies among other histopathological classes.

In conclusion, the present study demonstrated that ATG5 represents a reliable autophagy-related biomarker in proliferative LN, with meaningful predictive utility for treatment outcomes. Reduced intra-renal ATG5 expression scores at baseline are associated with favourable therapeutic responses, particularly in patients receiving MMF as induction therapy. These findings support the incorporation of ATG5 assessment both in serum and renal tissue into the diagnostic and monitoring framework of LN, pending validation in larger prospective cohorts.

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Author Contributions

All authors certify that they participated in this work including participation in study design, collecting data, analysis and interpretation of data, practical part and writing manuscript.

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Ethical approval

The study protocol was reviewed and approved by the Institutional Ethics Committee of the Faculty of Medicine, Ain Shams University (approval number: FWA 000017585).

Informed consent

A written informed consent was obtained from each participant prior to their inclusion in the study.

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