American Journal of Biochemistry 2018, 8(2): 19-29
DOI: 10.5923/j.ajb.20180802.01

Association of Interleukin 23 and Anti-ficolin -2 Antibodies with Lupus Nephritis

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Abstract **Objective:** To analyzed the serum concentration of Anti-ficolin 2 antibodies and Interleukin 23 in SLE patients and to evaluate the association between their levels with disease activity and also degree of lupus nephritis. **Patients and methods:** The present study included 90 subjects, 70 SLE patients (40 patients with lupus nephritis (LN), 30 SLE patients without LN), and 20 age and sex matched healthy control (HC). An enzyme-linked immunosorbent assay (ELISA) was used to analyze the serum concentration of Anti-ficolin 2 antibodies and IL 23. The correlations between the levels of Anti-ficolin 2 antibodies, IL-23, and disease activity, clinical parameters in SLE patients were analyzed. **Results:** Levels of IL-23 and anti ficolin-2 auto antibodies were significantly higher in patients with SLE compared with healthy controls, and in patients with SLE and renal involvement (LN patients) compared with SLE alone (Non LN patients). Patients with proliferative LN had significantly more IL23 and anti-ficolin-2 antibodies than those with non-proliferative LN. Anti-ficolin-2 antibodies were associated with the SLEDAI score. **Conclusion:** Our findings suggest that IL-23 and anti-ficolin-2 may be useful biomarkers for the diagnosis of SLE with renal manifestation.

Keywords Interleukin 23, Anti ficolin -2 antibodies, Lupus nephritis

1. Introduction

Systemic lupus erythematosus is a chronic autoimmune disease that can affect virtually any organ of the body. [1] Systemic lupus erythematosus presentation and course are highly variable, ranging from indolent to fulminant course. In childhood onset SLE, there are several clinical symptoms more commonly than adult, Raynaud's, pleuritis and sicca are twice as common as in children and adolescents [2]. The diagnosis of SLE is based on a combination of clinical findings and laboratory investigation. The Presence of 4 of the 11 criteria of American College of Rheumatology (ACR) criteria yields a sensitivity of 85% and specificity of 95%for SLE [3].

Systemic Lupus Erythematosus is characterized by presence of circulating auto antibodies directed against self antigen as ds DNA, nuclear antigen and several cytoplasmic components [4]. The accumulation of the resulting immune complex mediates a systemic inflammatory response that is the primary cause of tissue damage and consequently defects in apoptotic cell clearance leading to secondary cell necrosis and subsequent release of intracellular autoantigens has been proposed as one of the mechanisms of induction of these auto antibodies [5].

Lupus nephritis (LN) is a severe manifestation of systemic lupus erythematosus (SLE), affecting up to 60% of SLE patients at some point of the disease [6]. The pathogenesis for LN is complex and involves multiple components of both the innate and adaptive immune systems. Although the exact mechanisms remain unclear, the hallmark in the pathogenesis is production of auto antibodies and immune complex formation with subsequent infiltration by inflammatory cells in renal tissue [7]. Many cytokines are also involved and contribute to both onset and progression of renal pathology. Complement components deficiency are often associated with increased susceptibility of both infections and autoimmune disease as SLE, human studies report that hereditary homozygous deficiencies of c1q are strongly associated with susceptibility of SLE with defect in apoptotic cells uptake by macrophage in SLE [8]. Also there is a role for mannan –binding lectin (MBL), a recognition protein of the lectin complement pathway in the pathogenesis of SLE [9] Ficolin-2 is an oligomeric protein

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composed of 35 kDa subunits assembled in triple helix, consisting of both collagen-like and fibrinogen-like domains. It is synthesized in the liver and secreted into the bloodstream where it is one of the major pattern recognition molecules of plasma/serum. Ficolin-2 plays an important role in innate immunity and the maintenance of tissue homeostasis due to its capacity to bind specific pathogen- and apoptotic cells-associated molecular patterns receptors. Target recognition triggers the innate immune response by either activating the lectin complement pathway or binding to collectin cellular receptors [10]. Antibodies against several complement recognition proteins, including C1q, mannose-binding lectin (MBL) and ficolin-3, have been reported to contribute to the development of SLE, supporting the hypothesis that the complement system is deeply involved in the pathogenesis of this disease in multiple ways [11].

Interleukin- (IL-) 23 belongs to the IL-12 family cytokines and consists of two subunits, p19 and p40. IL-23 is produced by macrophages, dendritic cells, keratinocytes, and other antigen-presenting cells and through its interaction with the IL-23 receptor plays a central role in inflammation including the induction of Th17 cells [12]. The IL-23-IL-17 axis is emerging as a critical regulatory system that bridges the innate and adaptive arms of the immune system and plays a critical role in development of autoimmune inflammatory diseases [13]. Previous studies found high serum levels of IL-23 in LN and an expansion of T-cells expressing both high IL-17 and IL-23-receptor have been demonstrated in lupus-prone mice. IL-23–treated lymphocytes have been shown to induce nephritis in mice, while IL-23- receptor deficiency has been found to prevent development of nephritis in murine lupus models [14].

2. Patients and Methods

This study was conducted between April 2017 and December 2017 in Al zahraa university hospital. Informed consent was obtained from all patients. We recruited two groups of SLE patients, 40 (34 women and 6 men; mean age 33.30 ± 13.84years) patients with renal involvement (the LN group) and 30 (28 women and 2 men; mean age 32.73 ± 10.11years) patients with no history of renal disease (the non-LN group). All patients fulfilled the updated revised American College of Rheumatology (ACR) classification criteria for SLE [15] SLE renal involvement was defined as an increase in protein/Creatinine ratio (> 0.5 g/g creatinine).

Renal biopsy-proven LN specimens were evaluated according to the classification criteria defined by the International Society of Nephrology/Renal Pathology Society (ISN/RPS) [16]. We also recruited 20 healthy controls (HC; 12 women and 8 men; mean age 39.25 ± 10.01years).

The following data were recorded: age, gender, disease duration, activity of the disease according to Systemic Lupus Erythematosus Activity Index (SLEDAI) [17]. Blood samples were obtained for determination of the complete blood cell count, serum Creatinine, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum complement C3, C4, and anti-double stranded DNA (dsDNA) antibody. A complete urinalysis was performed and the ratio of urinary protein to urinary creatinine (Upro: Ucre) was obtained. Serum samples from each patient or control subject were frozen at _20°C for later analysis of anti ficolin-2 antibody and IL23.

Complete blood count was determined by cell counter sysmex KX21N (Germany) using kits of siemens (Germany). serum creatinine was done using Cobas C311 (Germany) and kits of Roche (Germany). CRP was detected by turbidmetric technique supplied from Biosystem (spain) with Lot number 19420; using 8897 photpmeter 5010; Germany. Serum complement C3, C4 were determined by Radial immunodiffusion method (DIFFU-PLATE) from Biocientifica S.A. (Argentina). Anti-double stranded DNA (dsDNA) antibody was determined by indirect immunofluoresence technique using immunofluoresence microscope (ZEISS-Axiolabe-Ser. Nr. 994869) and the kit was supplied from Diasorin (USA) with Lot number 133670. Anti-ficolin-2 antibodies was measured by quantitative Enzyme Linked immunosorbent Assay supplied from Bioassay Technology Laboratory with Cat. No E0330Hu (Standard curve Range: 2ng/ml – 600ng/ml and sensitivity: 1.01 ng/ml); using AS 1851 Das; italy (reader) and 16041412 Bio Tek; USA (washer). Serum IL23 concentration was measured by quantitative sandwich enzyme immunoassay technique supplied from Glory science Co., Ltd with Lot No: 2017119 (detection range is 15 ng/L – 400ng/L); using AS 1851 Das; italy (reader) and 16041412 Bio Tek; USA (washer).

Statistical Analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges when their distribution found parametric while with non parametric data were presented as median with inter-quartile range (IQR). Also qualitative data were presented as number and percentages. The comparison between two independent groups with qualitative data was done by using Chi-square test and/or Fisher exact test only when the expected count in any cell found less than 5. The comparison between two independent groups with quantitative data and parametric distribution was done by using Independent t-test while with non parametric data were done by using Mann-Whitney test. The comparison between more than two independent groups with quantitative data and parametric distribution was done by using One Way ANOVA test while with non parametric data were done by using Kruskall-Wallis test. Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group. Receiver operating characteristic curve (ROC) was used to assess the best cut off point with its sensitivity, specificity, positive

predictive value (PPV), negative predictive value (NPV) and area under curve (AUC). The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant at the level of < 0.05.

3. Results

3.1. Clinical Characteristics of SLE Patients

A total of 70 SLE patients (40 with LN and 30 without LN) were recruited to this study and their clinical characteristics are shown in Table 1. Renal biopsies were performed in 22 patients with LN, one patient with Class I, 6 patients with class II LN, 3 patients with class III, LN 10 patients with class IV LN, and 2 patients with class V LN. There were significant differences in anti-dsDNA antibody levels, C3, C4, and SLEDAI score between SLE patients with and without LN.

3.2. Serum Levels of IL-23 and Anti-ficolin -2 Antibodies

Serum levels of IL-23 and anti-ficolin -2 were significantly higher in LN patients (mean 109.06 ng/L and 86.28 ng /mL, respectively) than in non-LN SLE patients (mean 42.72 ng/L and 55.99 ng /mL, respectively; all $p < 0.05$) and HC (23.04 ng/L and 26.30 ng /mL, respectively; all $p < 0.05$). In addition, there was significant difference in serum IL-23 and anti-ficolin -2 levels between non-LN patients and HC ($p < 0.001$). Table (1,2)

Renal biopsy-proven LN specimens were evaluated in 22 LN patients. Significantly higher serum levels of IL-23 and anti-ficolin -2 were observed in patients with class III, class IV LN (proliferative) than in patients with class I, II, and class V LN patients (Non proliferative) Table (3)

Table 1. Demographic data and laboratory results of SLE patients

		Non LN group	LN group	Test value	P-value
		No. = 30	No. = 40		
Age	Mean±SD	32.73 ± 10.11	33.30 ± 13.84	0.189	0.850
	Range	17 – 52	14 – 62		
Gender	Females	28 (93.3%)	34 (85.0%)	1.176	0.278
	Males	2 (6.7%)	6 (15.0%)		
DD	Mean±SD	3.30 ± 1.84	3.95 ± 2.31	1.268	0.209
	Range	1 – 7	1– 10		
SLEDAI score	Mean±SD	4.73 ± 2.15	7.03 ± 1.33	5.494	0.000
	Range	2 – 9	5 – 13		
Biopsy	G I	0 (0.0%)	1 (4.5%)	NA	NA
	G II	0 (0.0%)	6 (27.3%)		
	G III	0 (0.0%)	3 (13.6%)		
	G IV	0 (0.0%)	10 (45.5%)		
	G V	0 (0.0%)	2 (9.1%)		
IL23 (ng/L)	Mean±SD	42.72 ± 15.91	109.06 ± 36.40	9.324	0.000
	Range	23 – 75.1	61.5 – 189		
Anti-Ficolin 2 antibody (ng/ml)	Mean±SD	55.99 ± 22.31	86.28 ± 27.16	4.974	0.000
	Range	22 – 97	47.3 – 160		
Protein/creatinine ratio (g/g)	Mean±SD	0.03 (0.02 – 0.04)	1.34 (0.8 – 3.6)	5.190	0.000
	Range	0.01 – 0.49	0.55 – 9.8		
Serum creatinine	Mean±SD	0.72 ± 0.29	2.17 ± 2.24	3.506	0.001
	Range	0.44 – 1.67	0.44 – 11.19		
CRP (mg/L)	Mean±SD	5 (4 – 6)	6 (4.5 – 19)	1.665	0.100
	Range	3 – 96	3 – 280		
DNA	Negative	11 (36.7%)	7 (17.5%)	3.267	0.069
	Positive	19 (63.3%)	33 (82.5%)		
C4 (mg/dl)	Mean±SD	30.92 ± 10.72	15.41 ± 7.24	7.222	0.000
	Range	18.4 – 64.6	2.4 – 37.4		
C3 (mg/dl)	Mean±SD	115.04 ± 31.09	76.79 ± 20.22	6.229	0.000
	Range	70.9 – 169.1	36.5 – 122.7		
ESR (mm/h)	Mean±SD	32 (20 – 60)	40 (23 – 57.5)	1.111	0.270
	Range	3 – 85	13 – 90		

DD:Disease duration

Table 2. Comparison between LN, Non LN and controle groups regarding demographic data and laboratory results

		Control group	Non LN group	LN group	One Way ANOVA		Post hoc anlaysis		
		No. = 20	No. = 30	No. = 40	F	P-value	P1	P2	P3
Gender	Females Males	12 (60.0%) 8 (40.0%)	28 (93.3%) 2 (6.7%)	34 (85.0%) 6 (15.0%)	21.921	0.000	0.000	0.000	0.278
Age	Mean±SD Range	39.25 ± 10.01 23 – 55	32.73 ± 10.11 17 – 52	33.30 ± 13.84 14 – 62	2.123	0.126	0.061	0.071	0.844
IL23 (ng/L)	Mean±SD Range	23.04 ± 6.16 15 – 35	42.72 ± 15.91 23 – 75.1	109.06 ± 36.40 61.5 – 189	92.506	0.000	0.011	0.000	0.000
Anti-Ficolin 2 antibody (ng/ml)	Mean±SD Range	26.30 ± 4.66 18 – 34	55.99 ± 22.31 22 – 97	86.28 ± 27.16 47.3 – 160	49.940	0.000	0.000	0.000	0.000
CRP (mg/L)	Median (IQR) Range	4.5 (3.5 – 5.5) 3 – 8	5 (4 – 6) 3 – 96	6 (4.5 – 19) 3 – 280	11.043	0.004	0.325	0.002	0.020
ESR (mm/h)	Median (IQR) Range	8 (6 – 12) 4 – 20	32 (20 – 60) 3 – 85	40 (23 – 57.5) 13 – 90	38.715	0.000	0.000	0.000	0.287

Chi-square test; one way ANOVA test

Table 3. Comparison between proliferative and non proliferative lupus nephritis regarding serum level of IL 23 & anti ficolin 2 antibodies

		Non Proliferative	Proliferative	Test value	P-value
IL23 (ng/L)	Mean±SD Range	91.54 ± 14.83 61.5 – 114	153.69 ± 28.12 100 – 189	6.043	0.000
Anti-Ficolin 2 antibody (ng/ml)	Mean±SD Range	74.60 ± 14.00 62.7 – 99	95.82 ± 25.07 68.6 – 144	2.292	0.033

Chi-square test; Independent t-test

Table 4. Correlation of IL-23 and anti-ficolin 2 with the other studied parameters in non LN group

Non LN patients	IL23 (ng/L)		Anti-Ficolin 2 (ng/ml)	
	r	p-value	r	p-value
Age	0.010	0.957	-0.111	0.559
DD	0.231	0.220	0.068	0.721
SLEDAI score	0.002	0.991	0.420*	0.021
Protein/creatinine ratio (g/g)	0.102	0.592	0.024	0.900
serum creatinine	0.024	0.902	-0.084	0.659
CRP (mg/L)	0.003	0.986	0.034	0.859
C4 (mg/dl)	-0.104	0.584	-0.498**	0.005
C3 (mg/dl)	0.017	0.931	-0.480**	0.007
ESR (mm/h)	-0.116	0.540	0.210	0.264

* Significant correlation
** Highly significant correlation

Table 5. Correlation of IL-23 and anti-ficolin 2 with the other studied parameters in LN group

LN patients	IL23 (ng/L)		Anti-Ficolin 2 antibody (ng/ml)	
	r	p-value	r	p-value
Age	-0.403**	0.010	0.103	0.527
DD	-0.002	0.992	0.072	0.659
SLEDAI score	0.001	0.995	0.365*	0.021
Protein/creatinine ratio (g/g)	0.388*	0.013	0.243	0.131
serum creatinine	-0.204	0.208	0.260	0.105
CRP (mg/L)	-0.165	0.308	-0.041	0.802
C4 (mg/dl)	0.037	0.820	-0.614**	0.000
C3 (mg/dl)	-0.043	0.791	-0.411**	0.008
ESR (mm/h)	0.092	0.574	-0.049	0.765

* Significant correlation
** Highly significant correlation

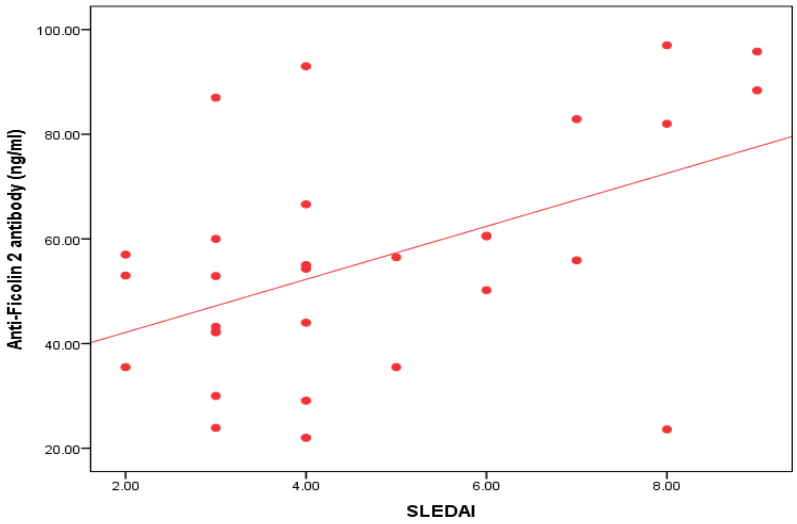


Figure 1. Correlation betweenserum level of Anti ficolin 2 antibodies and SLEDAI score in Non LN

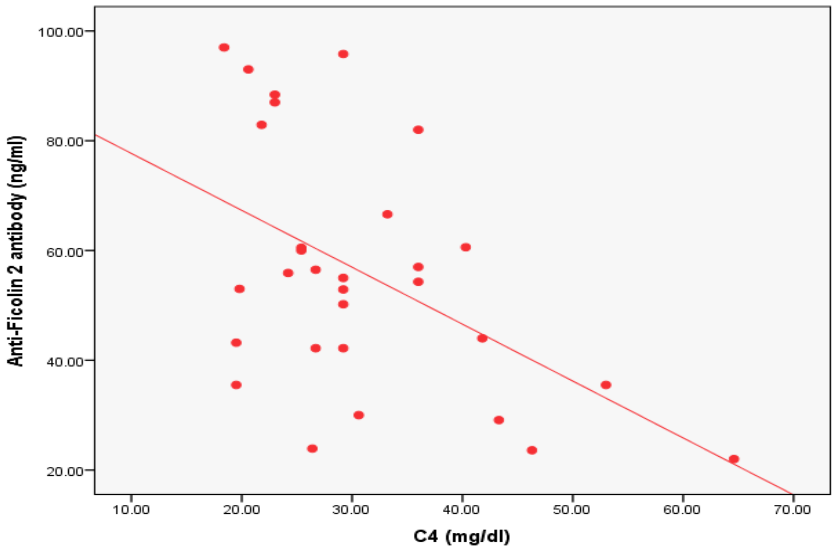


Figure 2. Correlation between serum level of Anti ficolin 2 antibodies and C4 in Non LN

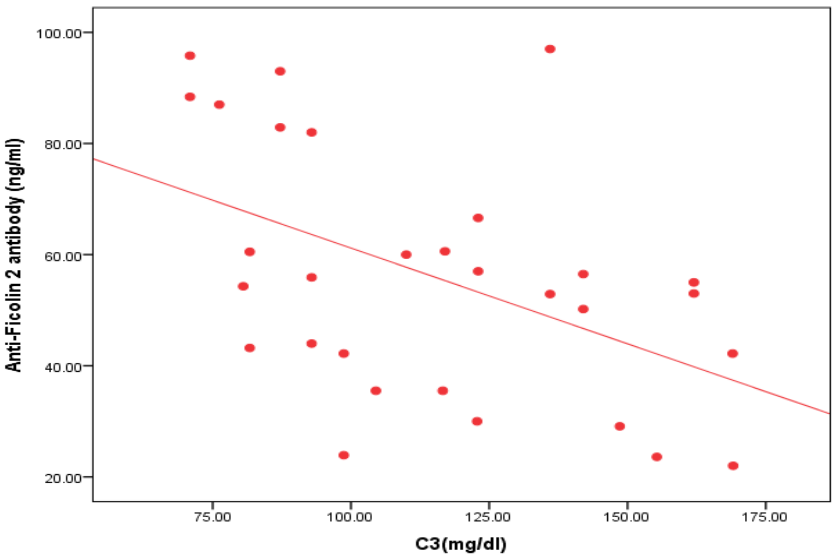


Figure 3. Correlation between serum level of Anti ficolin 2 antibodies and C3 in Non LN

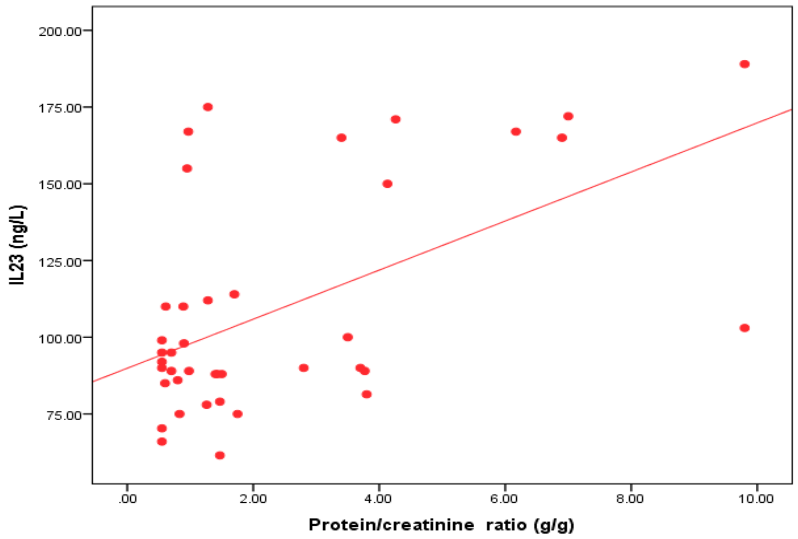


Figure 4(A). Correlation between serum level of IL 23 and protein /creatinine Ratio in LN

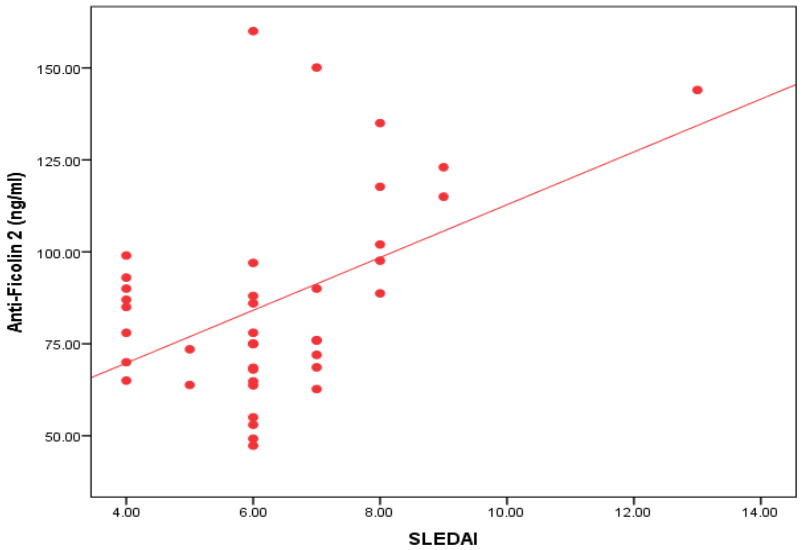


Figure 4(B). Correlation between serum level of Anti ficolin 2 antibodies and SLEDAI score in LN

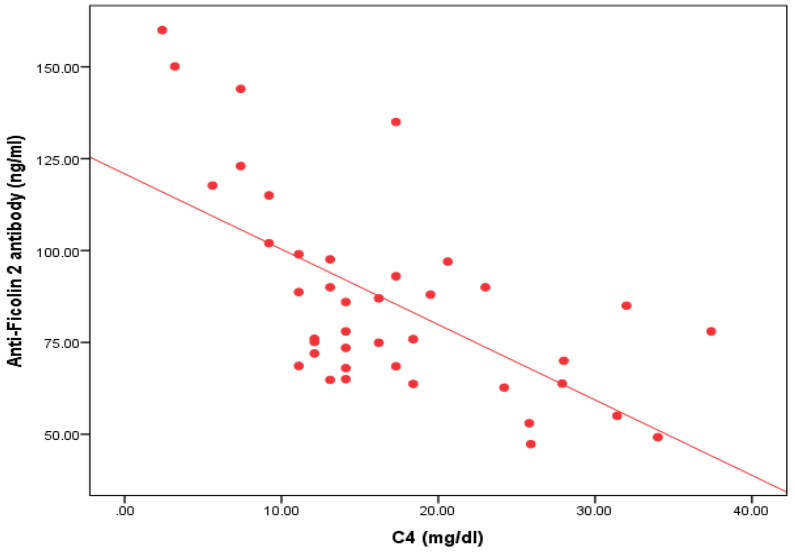


Figure 4(C). Correlation between serum level of Anti ficolin 2 antibodies and C4 in LN

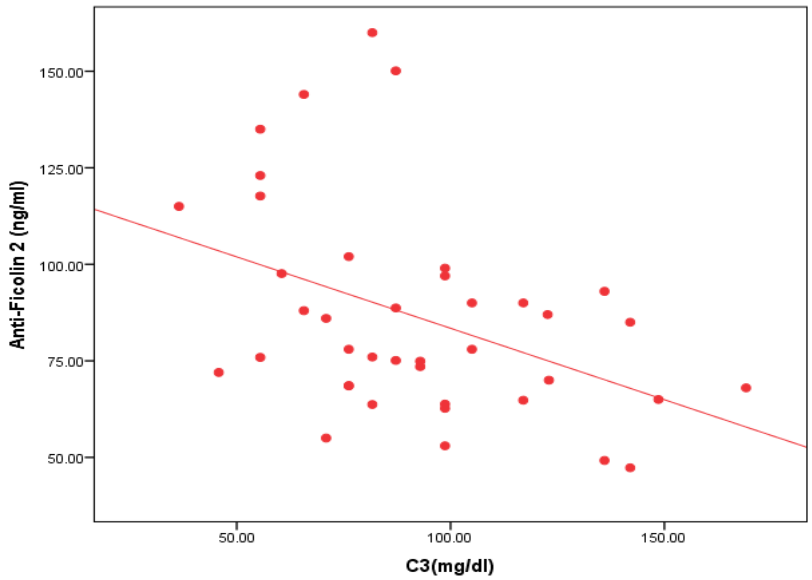


Figure 4(D). Correlation between serum level of Anti ficolin 2 antibodies and C3 in LN

Table 6. Relation of anti-ficolin 2 antibodies with DNA

Groups	DNA	Anti-Ficolin 2 antibody (ng/ml)		Test value	P-value	Sig.
		Mean ± SD	Range			
Non LN	Negative (n = 11)	48.42 ± 15.96	23.9 – 93	2.163	0.039	S
	Positive (n = 19)	62.91 ± 18.57	22 – 97			
LN	Negative (n = 7)	77.57 ± 11.56	68 – 99	2.085	0.044	S
	Positive (n = 33)	93.64 ± 19.55	47.3 – 160			

S: significant

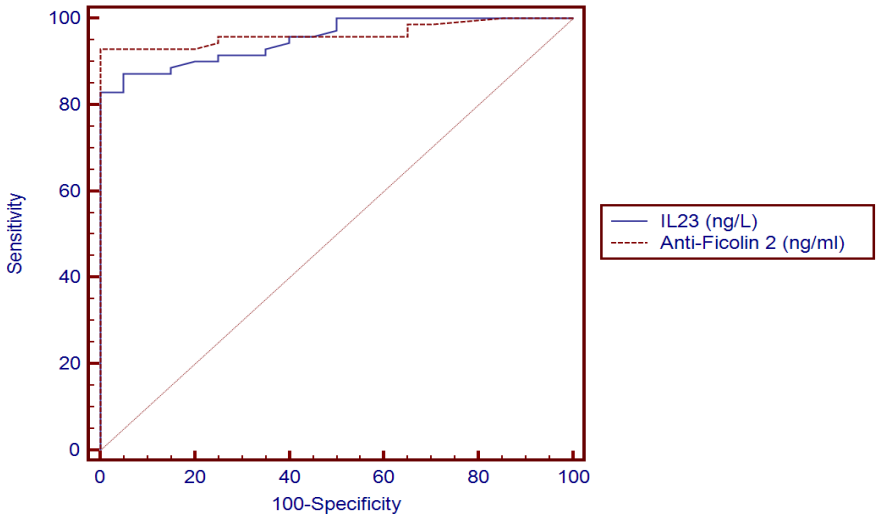


Figure 5. ROC curve for IL-23 and anti ficolin-2 in differentiation between SLE cases and control

Table 7. ROC curve for IL-23 and anti ficolin-2 in differentiation between SLE cases and control

	Cut off point	AUC	Sensitivity	Specificity	PPV	NPV
IL23 (ng/L)	>35	0.952	82.86	100	100	62.5
Anti Ficolin-2 (ng/ml)	>34	0.964	92.86	100	100	80

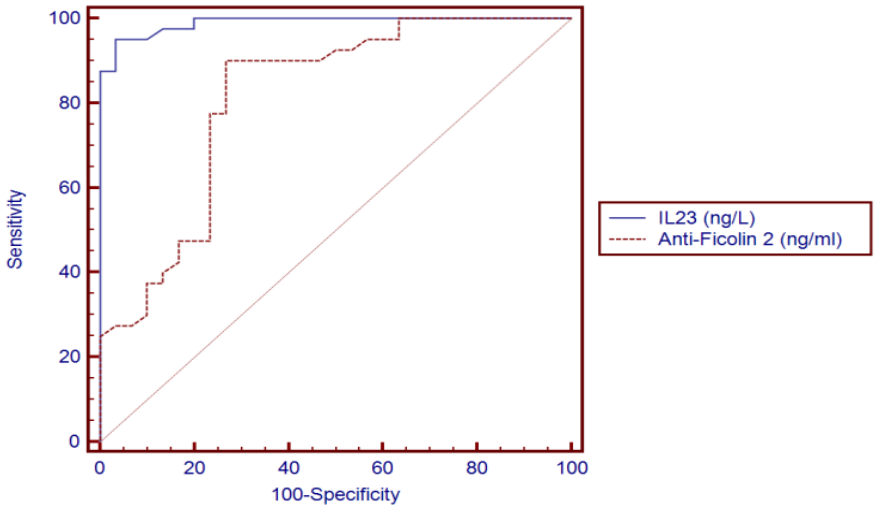


Figure 6. ROC curve for IL-23 and anti ficolin-2 in differentiation between patients with LN and Non LN

Table 8. ROC curve for IL-23 and anti ficolin-2 in differentiation between patients with LN and Non LN

	Cut off point	AUC	Sensitivity	Specificity	PPV	NPV
IL23 (ng/L)	>70	0.99	95	96.67	97.4	93.5
Anti Ficolin-2 (ng/ml)	>60.6	0.814	90	73.33	81.8	84.6

3.3. Correlations of IL-23 and Anti-ficolin -2 Antibodies with Disease Activity and Clinical Parameters in SLE Patients

In the non-LN SLE patients, although elevated level of IL-23 in the serum was found, there was no significant correlations with disease activity (SLEDAI score) and other clinical parameters. While in LN SLE patients there was a positive correlation with protein /creatinine Ratio (r = 0.388*, p = 0.013) Table (4, 5) Figure 4. (A)

A positive correlation between anti-ficolin-2 antibodies titers and lupus activity using SLEDAI score was observed (in Non LN and LN) (r =0.420*, p = 0.021), (r =0.365*, p = 0.021) respectively. A Significant negative correlation was found between anti-ficolin-2 antibodies and C4, C3 in SLE patients (Non LN and LN)(r = - 0.498**, p =0.005) (r = - 0.614**, p = 0.000) (r = - 0.480**, p =0.007) (r = - 0.411**, p = 0.008) respectively. Table (4, 5) Figures (1, 2, 3, 4 B, C, D).

In SLE patients there was statistically significant increase in the level of Anti-ficolin 2 antibody in patients with positive DNA than those with negative DNA (in non LN and LN patients) with p-value = 0.039 and 0.044 respectively. Table (6)

Titers of anti-ficolin-2 antibodies were significantly higher in SLE patients than in healthy controls Using a cutoff point of >34 with Sensitivity and specificity 92.86 and 100 respectively. Table (7) Figure (5)

Sensitivity and specificity of anti-ficolin-2 antibodies for SLE renal involvement were respectively 90% and 73.3%.

Predictive positive and negative values were respectively 81.8% and 84.6%. Table (8) Figure (6)

4. Discussion

Systemic lupus erythematosus is a highly heterogeneous disease. Characterization of subgroups of patients with SLE into specific disease phenotypes, such as those with major organ involvement, may facilitate the investigation of disease pathogenesis. For example, lupus nephritis affects over 50% of patients with SLE and is a major cause of morbidity [18] Despite significant advances in the understanding of interactions between immune cells, soluble factors and resident kidney cells, the precise pathophysiological mechanisms that underlie the development of lupus nephritis remain unclear [19]. Although the aetiopathogenesis of LN remains to be elucidated, previous studies have focused on T-cell homeostasis [20, 21]. SLE is often considered to be a Th2-mediated disease in the early stage [22], but the Th1commitment is only substituted for the Th2 pathway and takes over the progression of SLE to active nephritis [23]. Both Th17 cells and regulatory T cells (Tregs) have been shown to participate in the pathopoiesis of SLE [24]. IL-23 is a pro-inflammatory cytokine that is indispensable in Th17 development mediated by stabilizing the Th17 phenotype and IL-17 expression [25, 26]. Evidence for the importance of IL-23 in the pathogenesis of LN has been introduced [27, 28]. Our results show that serum IL-23 levels were significantly higher in SLE patients than in HC, which is consistent with the findings of previous studies [29, 30]. Our study further supports the role of IL-23 in the pathogenesis of LN, and provides evidence that serum IL-23 could be a candidate clinical biomarker for LN. Nevertheless, further investigations are needed to determine the exact role of

IL-23 in the pathogenesis of LN. In summary, we found an increase in level of IL-23 in serum in LN patients compared with non-LN SLE patients and HC, and there was also an important correlation of IL-23 serum concentrations and lupus nephritis, which is in accordance with other data. The relationship between the elevated serum levels of IL-23 and renal involvement in SLE patients was confirmed by several studies [31-33]. High serum IL-23 mRNA levels were also found in patients with SLE and renal disease [34]. These results suggest that IL-23 is involved in the pathogenesis of renal disease in SLE and, as such, could represent a possible biomarker for renal involvement in SLE and a therapeutic target in this subgroup of patients. Published research has demonstrated that the IL-23/IL-17 pathway is activated in lupus-prone mice and is associated with increased immunoglobulin deposition and complement activation in the kidney, [35] further indicating an important role for IL-23 in SLE renal pathology.

We did not confirm the relationship between SLE activity and other clinical characteristics of the disease. In the literature, contrary data are available on the association of IL-23 and SLE activity. Some reports showed a correlation between high levels of IL-23 and activity of the disease [36] as well as a relationship between IL-23 levels and selected manifestations like serositis and cutaneous involvement among patients with active disease [37]. Some reports presented discrepant results and did not show any association of serum IL-23 and SLE activity [38-40]. In our study, there is lack of a relationship between IL-23 and activity of SLE. in our study we did not find any correlation of IL- 23 with auto antibodies typical for lupus like anti-double stranded DNA which is consistent with the findings of other studies [36, 37]. As biomarkers currently available for assessing renal activity or response to therapy do not always reflect the actual inflammatory activity in renal tissue [41-43] there is a clear need for novel tools to assess activity in LN. Although new biomarkers both in blood and urine, including several cytokines, have been studied in LN, none have yet replaced conventional clinical parameters to monitor renal disease activity in clinical practice. It has also been proposed that a combination of biomarkers should be used to provide the best specificity and sensitivity [44-46]. Our findings of high IL-23 in LN suggest that these cytokines could be evaluated as novel biomarkers for severe disease. Increased knowledge about the pathogenesis for SLE, in combination with the successful use of biologics in other autoimmune diseases, have paved the way for the development of biologic agents targeting different cytokine pathways also in SLE and thus, many anti-cytokine therapies for SLE are currently studied [47]. Trials targeting the IL-23/IL-17 pathway are underway for various autoimmune diseases, and targeting IL-23 (p40) is already in use for psoriasis, but the efficacy of these therapies in lupus remains to be studied [48]. Interestingly, a recent report documented beneficial effects of treatment of subacute cutaneous lupus with ustekinumab, a human monoclonal antibody that binds to the p40 subunit of IL- 23 and inhibits its biological effects [49], indicating that blocking of IL-23 may be useful in lupus patients.

In Our study we also assess the presence of antibodies targeting ficolin-2 and measuring their titers. Levels of anti-ficolin-2 auto antibodies were significantly higher in SLE patients compared to healthy subjects and a positive correlation of anti-ficolin-2 antibodies with the SLEDAI score which reflects the disease activity was found, the presence of anti-ficolin-2 antibodies was significantly related to renal involvement as we observed a high prevalence of anti-ficolin-2 antibodies in the subset of SLE patients with active LN and this in consistent with a pervious study [50]. Lupus nephritis, one of the most frequent and serious complications in SLE patients, and a major predictor of poor outcome, is a real challenge in the management of SLE. In clinical practice, conventional parameters such as proteinuria, abnormal creatinine and an elevated anti-dsDNA antibody titer concomitant with reduced complement levels (C3, C4) are disease-activity biomarkers of SLE and LN. However, they still lack sensitivity and specificity for detecting ongoing disease activity in lupus kidneys and early relapse of nephritis [51]. Our results indicate that anti-ficolin-2 antibodies might be an additional parameter to distinguish active LN from active non-renal SLE flare. A significant correlation between anti-ficolin-2 antibodies and other SLE biological biomarkers was found, including anti-dsDNA antibodies, low complement levels, anti-C1q antibodies and also anti-ficolin-3 antibodies, was reported to be significantly associated with active LN [52]. So the combination of anti-C1q, anti-ficolin-3 and anti-ficolin-2 could open new perspectives for LN evaluation, with future directions in LN biomarker research focused on new multi-marker panels, including these antibodies. A high negative predictive value of anti-ficolin-2 antibodies for nephritis was found as reported for anti-C1q antibodies [53]. It is interesting in the present study, that patients with proliferative LN (i.e. Classes III and IV) showed significantly more positive anti-ficolin-2 antibodies than those with non-proliferative LN. Although the relationship between the presence of anti-ficolin-2 antibodies and LN remains to be determined, the possible role of anti-ficolin-2 antibodies in the pathogenesis of LN and its activity is a key issue. The anti-ficolin-2 antibodies would affect the occurrence or the course of SLE disease by interfering with ficolin-2 functions, especially with apoptotic cells clearance, or by binding to ficolin-2 deposited on various tissues. It has been previously demonstrated that ficolin-2 is deposited in the glomerular tissue in patients with LN together with IgG, C3, C1q, terminal complement cascade components and MBL [54]. Ficolin-2 could be a key component in tissue inflammation as a target for auto antibodies. Anti-ficolin-2 antibodies may contribute to the formation of circulating immune complexes that are deposited in the kidney or to the local formation of immune complexes at the level of glomerular basement membrane, leading to complement activation and subsequent tissue injury [53].

5. Conclusions

In conclusion, the present study suggests the involvement of IL-23 in SLE pathogenesis. Serum IL-23 levels were significantly elevated in patients with SLE compared with healthy controls, and in patients with SLE and renal disease compared with patients without renal involvement. Our finding also supports the previous data of presence of anti-ficolin-2 antibodies in the serum of SLE patients with presence of higher levels in patients with lupus nephritis. These data support the usefulness of IL 23 and anti-ficolin-2 as a potential biomarkers for the diagnosis of active lupus with renal manifestation. Further studies with larger number of patients are necessary to validate the diagnostic and/or prognostic role of these new SLE parameters.

REFERENCES

[1] Crowson Cs, Matteson EL, Maysoedova E, etal. The life time risk of adults onset rheumatoid arthritis and other inflammatory autoimmune rheumatic diseaseas. Arthritis Rheum. 2011; 63(3): 633-639.

[2] Livingston B, Bonner A, pope J. Differences in clinical manifestations between childhood-onset lupus and adult-onset lupus; a metanalysis Lupus. 2011 Nov. 20(13): 1345-55.

[3] American College of Rheumatology 1997 update of the 1982 American Collage of Rheumatology revised criteria for classification of SLE. Available at http;//tinyurl.com/1997 SLE criteria.sep.25, 2015.

[4] Arbuckle MR, Mc Clain MT, Rubertone MV, Scofield RH, Denn, s GJ, James JA, et al. Development of autoantibodies before the clinical onset of SLE.N. Engl. J Med. 2003; 349; 1526-1533.

[5] Munoz LE, Lauberk, Schiller M, Manfredi AA, Herrmann M. The role of defective clearance of apoptotic cells, systemic autoimmunity. Nat Rev. Rheumatol. 2010; 280-289.

[6] Singh S, Saxena R. Lupus nephritis. Am J Med Sci. 2009; 337(6): 451–60.

[7] Nowling TK, Gilkeson GS. Mechanisms of tissue injury in lupus nephritis. Arthritis Res Ther. 2011; 13(6): 250.

[8] Botto M, Walprt MJ. C1q, autoimmunity and apoptosis. Immunobiology. 2002; 205: 395-406.

[9] Tstutsumi A, Takahashi R, Sumidat. Mannose binding lectin: genetics and autoimmune disease Autoimmune Rev. 2005; 4364-372.

[10] M. Matsushita, "The ficolin family: an overview," in Collagen-Related Lectins in Innate Immunity, D. Kilpatrick, Ed., pp. 17–31, Research Signpost, 2007.

[11] Plawecki M, Lheritier E, Clavarino G, Jourde-Chiche N, Ouili S, Paul S, et al. Association between the Presence of Autoantibodies Targeting Ficolin-3 and Active Nephritis in Patients with Systemic Lupus Erythematosus. PloS One. 2016; 11(9): e0160879.

[12] E. Duvallet, L. Semerano, E. Assier, G. Falgarone, and M. C. Boissier, "Interleukin-23: a key cytokine in inflammatory diseases," Annals of Medicine, vol. 43, no. 7, pp. 503–511, 2011.

[13] Y. Iwakura and H. Ishigame, "The IL-23/IL-17 axis in inflammation," The Journal of Clinical Investigation, vol. 116, no. 5, pp. 1218–1222, 2006.

[14] Kyttaris VC, Zhang Z, Kuchroo VK, Oukka M, Tsokos GC. Cutting edge: IL-23 receptor deficiency prevents the development of lupus nephritis in C57BL/6-lpr/lpr mice. J Immunol. 2010; 184(9): 4605–9.

[15] Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997; 40(9): 1725.

[16] Weening JJ, D'Agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, et al. The classification of glomerulonephritis in systemic lupus erythematosus revised. J Am SocNephrol 2004; 15: 241–50.

[17] C. Bombardier, D. D. Gladman, M. B. Urowitz et al., "Derivation of the SLEDAI. A disease activity index for lupus patients," Arthritis & Rheumatism, vol. 35, no. 6, pp. 630– 640, 1992.

[18] Balow JE. Clinical presentation and monitoring of lupus nephritis. Lupus 2005; 14: 25–30.

[19] Dimitriou SG and Madaio MP. Renal damage in systemic lupus erythematosus. In: Tsokos GC, Gordon C, Smolen J (eds) Systemic lupus erythematosus: a companion to rheumatology. Philadelphia, PA: Mosby, 2007, pp.287–292.

[20] Nakashima H, Akahoshi M, Masutani K. Th1/Th2 balance of SLE patients with lupus nephritis. RinshoByori 2006; 54: 706–13.

[21] Dolff S, Bijl M, Huitema MG, Limburg PC, Kallenberg CG, Abdulahad WH. Disturbed Th1, Th2, Th17 and T(reg) balance in patients with systemic lupus erythematosus. ClinImmunol 2011; 141: 197–204.

[22] Horwitz DA, Gray JD, Behrendsen SC, Kubin M, Rengaraju M, Ohtsuka K, et al. Decreased production of interleukin-12 and other Th1-type cytokines in patients with recent-onset systemic lupuserythematosus. Arthritis Rheum 1998; 41: 838–44.

[23] Akahoshi M, Nakashima H, Tanaka Y, Kohsaka T, Nagano S, Ohgami E, et al. Th1/Th2 balance of peripheral T helper cells in systemic lupus erythematosus. Arthritis Rheum 1999; 42: 1644–8.

[24] Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, et al. Adistinct lineage of CD4 T cells regulates tissue inflammation byproducing interleukin 17. Nat Immunol 2005; 6: 1133–41.

[25] Qu N, Xu M, Mizoguchi I, Furusawa J, Kaneko K, Watanabe K, et al. Pivotal roles of T-helper 17-related cytokines, IL-17, IL-22, and IL-23, in inflammatory diseases. Clin Dev Immunol 2013; 2013: 968549.

[26] Taherian M, Razavi AR, Izad M, Boghozian R, Namdari H, Ghayedi M, et al. The role of interleukin-23 in stability of in vitro T helper-17 cells. Iran J Allergy Asthma Immunol 2014; 13: 131–7.

[27] Zhang Z, Kyttaris VC, Tsokos GC. The role of IL-23/IL-17 axis in lupus nephritis. J Immunol 2009; 183: 3160–9.

[28] Kyttaris VC, Kampagianni O, Tsokos GC. Treatment with antiinterleukin 23 antibody ameliorates disease in lupus-prone mice.Biomed Res Int 2013; 2013: 861028.

[29] Qiu F, Song L, Yang N, Li X. Glucocorticoid downregulates expression of IL-12 family cytokines in systemic lupus erythematosus patients. Lupus 2013; 22: 1011–16.

[30] Mok MY, Wu HJ, Lo Y, Lau CS. The relation of interleukin 17 (IL-17) and IL-23 to Th1/Th2 cytokines and disease activity in systemic lupus erythematosus. J Rheumatol 2010; 37: 2046–52.

[31] D.-Y. Chen, Y.-M. Chen, M.-C. Wen, T.-Y. Hsieh, W.-T. Hung, and J.-L. Lan, "The potential role of Th17 cells and Th17-related cytokines in the pathogenesis of lupus nephritis," Lupus, vol. 21, no. 13, pp. 1385–1396, 2012.

[32] A. Zickert, P. Amoudruz, Y. Sundström, J. Rönnelid, V. Malmström, and I. Gunnarsson, "IL-17 and IL-23 in lupusnephritis - association to histopathology and response to treatment," BMC Immunology, vol. 16, no. 7, p. 10, 2015.

[33] L. P. Xia, B. F. Li, H. Shen, and J. Lu, "Interleukin-27 and interleukin-23 in patients with systemic lupus erythematosus: possible role in lupus nephritis," Scandinavian Journal of Rheumatology, vol. 44, no. 3, pp. 200–205, 2015.

[34] J. Du, Z. Li, J. Shi, and L. Bi, "Associations between serum interleukin-23 levels and clinical characteristics in patients with systemic lupus erythematosus," Journal of International Medical Research, vol. 42, no. 5, pp. 1123–1130, 2014.

[35] Zhang Z, Kyttaris VC and Tsokos GC. The role of IL-23/IL-17 axis in lupus nephritis. J Immunol 2009; 183: 3160–3169.

[36] D. Salah Hegab, M. Mahmoud Gamei, W. Mohamed Saudi, D. Ezzat Abou Ammo, M. Mohamed El Bedewy, and N. Fathy Elhabian, "Role of interleukin-23 in the immunopathogenesis of systemic lupus erythematosus," Egyptian Journal of Dermatology and Venerology, vol. 34, no. 2, pp. 120–125, 2014.

[37] M. Yin Mok, H. Jing Wu, Y. Lo, and C. Sing Lau, "The relation of interleukin 17 (IL-17) and IL-23 to Th1/Th2 cytokines and disease activity in systemic lupus erythematosus," The Journal of Rheumatology, vol. 37, no. 10, pp. 2046–2052, 2010.

[38] R. M. Talaat, S. F. Mohamed, I. H. Bassyouni, and A. A. Raouf, "Th1/Th2/Th17/Treg cytokine imbalance in systemic lupuserythematosus (SLE) patients: correlation with disease activity," Cytokine, vol. 72, no. 2, pp. 146–153, 2015.

[39] C. K. Wong, L. C. W. Lit, L. S. Tam, E. K. M. Li, P. T. Y. Wong, and C. W. K. Lam, "Hyperproduction of IL-23 and IL-17 in patients with systemic lupus erythematosus: implications for Th17-mediated inflammation in auto-immunity," Clinical Immunology, vol. 127, no. 3, pp. 385–393, 2008.

[40] X. Cheng, X. Yu, Y. J. Ding et al., "The Th17/Treg imbalance in patients with acute coronary syndrome," Clinical Immunology, vol. 127, no. 1, pp. 89–97, 2008.

[41] Gunnarsson I, Sundelin B, Heimburger M, Forslid J, van Vollenhoven R, Lundberg I, et al. Repeated renal biopsy in proliferative lupus nephritis– predictive role of serum C1q and albuminuria. J Rheumatol. 2002; 29(4): 693–9.

[42] Hill GS, Delahousse M, Nochy D, Remy P, Mignon F, Mery JP, et al. Predictive power of the second renal biopsy in lupus nephritis: significance of macrophages. Kidney Int. 2001; 59(1): 304–16.

[43] Lightstone L. Lupus nephritis: where are we now? CurrOpinRheumatol. 2010; 22(3): 252–6.

[44] Rovin BH, Zhang X. Biomarkers for lupus nephritis: the quest continues. Clin J Am SocNephrol. 2009; 4(11): 1858–65.

[45] Mok CC. Biomarkers for lupus nephritis: a critical appraisal. J Biomed Biotechnol. 2010; 2010: 638413.

[46] Adhya Z, Borozdenkova S, Karim MY. The role of cytokines as biomarkers in systemic lupus erythematosus and lupus nephritis. Nephrol Dial Transplant. 2011; 26(10): 3273–80.

[47] Ronnblom L, Elkon KB. Cytokines as therapeutic targets in SLE. Nat RevRheumatol. 2010; 6(6): 339–47.

[48] Davis LS, Hutcheson J, Mohan C. The role of cytokines in the pathogenesis and treatment of systemic lupus erythematosus. J Interferon Cytokine Res.2011; 31(10): 781–9.

[49] De Souza A, Ali-Shaw T, Strober BE, Franks Jr AG. Successful treatment of subacute lupus erythematosus with ustekinumab. Arch Dermatol. 2011; 147(8): 896–8.

[50] Colliard S, Jourde-Chiche N, Clavarino G, Sarrot-Reynauld F, Gout E, Deroux A, Fougere M, Bardin N, Bouillet L, Cesbron JY, Thielens NM, Dumestre-Pérard C. Autoantibodies targeting ficolin-2 in systemic lupus erythematosus patients with active nephritis. Arthritis Care Res (Hoboken). 2017 Oct 17. doi: 10.1002/acr.23449.

[51] Soliman S, Mohan C. Lupus nephritis biomarkers. ClinImmunol Orlando Fla. 2016; S1521-6616(16):30257-1.

[52] Plawecki M, Lheritier E, Clavarino G, Jourde-Chiche N, Ouili S, Paul S, et al. Association between the Presence of Autoantibodies Targeting Ficolin-3 and Active Nephritis in Patients with Systemic Lupus Erythematosus. PloS One. 2016; 11(9): e0160879.

[53] Trouw AL, Daha MR. Role of anti-C1q autoantibodies in the pathogenesis of lupus nephritis. Expert OpinBiolTher. 2005; 5(2): 243-51.

[54] Nisihara RM, Magrini F, Mocelin V, Messias-Reason IJ. Deposition of the lectin pathway of complement in renal biopsies of lupus nephritis patients. Hum Immunol. 2013; 74(8): 907-10.