Rzeszów University Press 2026
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2026.2.1

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2026; 24 (2): 254–262

ORIGINAL PAPER

Evaluating the roles of interleukin-17, C3, C4, antinuclear antibody, and antiphospholipid antibody in the pathophysiology of systemic lupus erythematosus

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ABSTRACT

Introduction and aim. Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by immune dysregulation, autoantibody (Abs) production, and complement (C) consumption. This study aimed to evaluate the diagnostic and prognostic relevance of antinuclear antibodies (ANA), interleukin (IL)-17A, complement components C3 and C4, and antiphospholipid (APL) Abs in patients to SLE compared with healthy individuals. This study provides an assessment of these biomarkers within a single cohort of an Iraqi population, addressing a regional knowledge gap.

Material and methods. A prospective case–control study was conducted in Al-Nasiriyah, Iraq, from July 2024 to July 2025, including 110 patients with SLE and 70 apparently healthy individuals. Serum levels of ANA, IL-17A, complement C3, complement C4 and APL immunoglobulin (Ig) M and G were measured using enzyme-linked immunosorbent assay (ELISA) and nephelometry. Statistical analyzes were included using descriptive statistics, chi-square tests, t-tests, and correlation analyses.

Results. Patients with SLE showed significantly higher levels of ANA, IL-17A, and APL Abs, along with significantly lower levels of complement C3 and C4. ANA was positively correlated with IL-17A and APL Abs and negatively with complement components. There were also significant negative correlations of APL-IgM/IgG with C3 and C4, while IL-17A did not show a correlation with C3 or C4.

Conclusion. These findings demonstrate coordinated immune activation and complement depletion in SLE and emphasize the value of combined biomarker evaluation within a context of a population of the Middle East specifically.

Keywords. antinuclear antibody, antiphospholipid antibody, autoimmunity, complement, interleukin 17, systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune chronic disease with multiple manifestations in many parts of the body, including inflammation, flares, and remission.[1] The fact that SLE is a complex and heterogeneous disorder has made it difficult for healthcare providers to find accurate and useful biomarkers to diagnose, manage, and targeting the treatment to improve outcomes for their patients with SLE.[2] Cytokine family interleukin (IL)-17A, complement components C3 and C4 and the antinuclear antibodies (ANA) and antiphospholipid (APL) antibodies (Abs) are all important markers of research interest relating to the evolving pathogenic mechanisms associated with SLE. IL-17 is derived from helper T cells and is primarily associated with inflammatory responses related to autoimmune diseases such as SLE.[3] Elevated levels of IL-17 were found in serum and tissues of SLE patients, and

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Received: 24.10.2025 / Revised: 13.12.2025 / Accepted: 4.01.2026 / Published: 30.06.2026

Khudhair HAA, Lafta SF. Evaluating the roles of interleukin-17, C3, C4, antinuclear antibody, and antiphospholipid antibody in the pathophysiology of systemic lupus erythematosus. *Eur J Clin Exp Med.* 2026;24(2):254–262. doi: 10.15584/ejcem.2026.2.1.

this elevation was found to correlate with both disease activity and severity. These observations suggest that IL-17 could be used as a clinical marker for lupus activity and response to treatment due to its ability to promote inflammation through direct activation of neutrophils and through the induction of other pro-inflammatory cytokines, and to promote a breakdown of body tolerance to its self-antigens.[4] However, it should be noted that several additional cellular and molecular markers have also been identified as having reasonable relevance to this classification of disease. These have been shown to be significant based on emerging studies that have been conducted with interest in direct or indirect relationships with basophils[5], double negative T cells[6], CD28-related pathways[7], and other auto-Abs[8] in the pathogenesis and/or activity/disease severity of SLE. Furthermore, the identification of these emerging markers adds context to the markers overall picture of the chosen in our study. SLE is also a heterogeneous disease that affects people of all ages, both adults and children, and thus, among age groups, the type of biomarker expressed and clinical significance may vary.[9] Therefore, when evaluating the potential clinical relevance of the specific biomarkers investigated in the current study, the broad spectrum of immunological processes and new biomarkers must also be considered.

Complement components C3 and C4 are central to immune defense and clearance of immune complexes. In SLE, consumption of C3 and C4, due to ongoing activation of the complement cascade, is a key characteristic of disease activity. Low levels of C3 and C4 levels are related to active disease flare-ups and, in particular, about lupus nephritis. Monitoring these complement components provides important information on disorder activity and treatment response.[10]

Because the ANA test has long been a component of evaluating patients for rheumatic disease, physicians and technicians should know the characteristics of the tests used. However, because novel test formats are used more frequently, research conducted in recent years has forced ANA testing to be re-evaluated. In addition, numerous novel medications are being tested in clinical trials to treat SLE, and these studies have revealed elements of ANA expression that are not visible through serological assays in a typical clinical environment. One of the most respected indicators in rheumatology, if not all medicine, is ANA; yet, the discipline is still evolving. Interpreting data from old procedures is often problematic, even though new techniques allow for enhanced depth and breadth of serological assays. However, the findings that the frequency of ANAs can influence treatment outcomes raise the possibility that these Abs have a valuable impact on the development of SLE in a way that is still not fully understood or recognized.[11]

Another significant classification of auto-Abs in SLE involves APL-Abs, which are indicative of the presence of antiphospholipid syndrome (APS) and may coexist with SLE. APL-Abs are directed against phospholipid-binding proteins that promote thrombosis and complications during pregnancy. The detection of APL-Abs is correlated with increased risk of vascular events and poor pregnancy outcomes in SLE. Given their association with the occurrence of SLE, APL-Abs are considered significant biomarkers and can identify people at risk for complications.[12] Interleukin-17, C3, C4, ANA, and APL-Abs are important biomarkers and provide information on the pathology of SLE and offer information to test, monitor, and managing SLE. The value of these biomarkers to reflect disease activity and to predict flares and outcomes provides clinical utility to use these markers in the management of SLE.

Although considerable research has been conducted on each of the individual components of the biomarkers mentioned above, little has been done to assess how the immunological interactions of multiple biomarkers from a given cohort work together. Furthermore, there is a lack of studies involving cohorts from the Middle East (especially Iraq).

Aim

Therefore, this study aims to address the lack of studies that simultaneously evaluate multiple immunological biomarkers by providing an integrated analysis of ANA, IL-17A, complement components C3 and C4, and APL-Ab in a cohort of systemic lupus SLE from Iraq. The study compares the levels between SLE patients and healthy controls (HC) and explores their interrelationships within the SLE group. This population-specific approach offers novel information on the coordinated immune pathways involved in the pathophysiology of SLE in a Middle Eastern population.

Material and methods

Participants and study design

This prospective case-control study was conducted in Al-Nasiriyah, Thi-Qar, Iraq, between July 2024 and July 2025. Patients were recruited from the rheumatology unit of Imam Al-Hussein Teaching Hospital, Al-Nasiriyah City. All participants were diagnosed with SLE by a consultant rheumatologist according to the 2019 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) classification criteria.[13] Consecutive patients who met the diagnostic criteria were enrolled using a convenience sampling approach after obtaining written informed consent.

The inclusion criteria comprised patients of either sex with a confirmed diagnosis of SLE and a disease duration of at least six months who were in regular follow-up at the same hospital. Exclusion criteria included

patients with coexisting autoimmune diseases, chronic metabolic or endocrine disorders, renal impairment not attributed to SLE, malignancy, and any acute or chronic infectious diseases.

Eligible patients were approached during their routine clinic visits and recruited after receiving a detailed explanation of the study protocol. Demographic and clinical information was obtained through structured interviews and review of medical records. Disease activity was not measured in this study, due to the fact that the current study focuses on immunological profiling rather than activity stratification. Treatments that included corticosteroids and hydroxychloroquine were listed and noted as part of descriptive statistical analyses to minimize bias from confounding variables. However, patients who were treated with high doses of corticosteroids or immunosuppressive pulse therapy and hydroxychloroquine within three months before participating in the study were not included in the analysis.

HCs were recruited from both community members and hospital employees. The HCs matched those diagnosed with SLE were of the same age, sex, and residence. The exclusion criteria for the subjects in this study were autoimmune disease, chronic illness, family history of SLE, immunosuppression, and infection.

Ethical approval

According to the principles stated in the Declaration of Helsinki, the research was approved on 21 July 2024 by the Medical Research Ethical Committee of Southern Technical University, Al-Nasiriyah Technical Institute, Iraq, with reference number 2742. Before beginning the study, informed consent was obtained from the patients for enrollment.

Sample size

The sample size was determined using the results of previous studies, the anticipated effect sizes, and the significance level of 0.05 with 80% power. To achieve this power, 46 participants per group were required, while there were actually 110 SLE subjects and 70 HC. Consequently, there was sufficient power to identify differences between the groups.

Measurement of immunological parameters

SLE immunodiagnostic assays including the following biomarkers: ANA status, APL-Abs (IgG and IgM) titers, complement levels (C3 and C4), and IL-17A level.

A 5 mL blood sample was placed at room temperature to coagulate for 30 minutes. The sample was centrifuged at 1500 g for 10 minutes to obtain serum. The serum was then aliquoted and placed in storage at -80°C until analysis could take place.

The serum ANA test was performed using an enzyme-linked ELISA technique utilizing a human ANA screen ELISA kit that was commercially prepared (Demeditec, Germany). According to the manufacturer's recommendations for the kit, the level of ANA in the serum sample was scaled as positive or negative, based on the optical density cut-off value. Less than 0.9 titers referred to the absence of ANA (ANA negative), while 0.9 titer was considered positive for ANA.

Serum APL-IgG and APL-IgM were performed by the ELISA technique using a phospholipid screen IgG/IgM ELISA Kit (Demeditec, Germany). The assay procedure was followed on the manufacturer's recommendations. According to the manufacturing cut-off levels, the concentrations of APL-IgG and APL-IgM were categorized as positive and negative, in which the level of <10 U/ml was considered negative, while the level of ≥10 U/mL was considered positive for both types of auto-Abs.

The complement C3 and C4 detection kit (Nephelometry) (Genrui, China) was used for serum C3 and C4 measurements. The assay procedure was followed according to the manufacturer's instructions, and the measured concentrations of C3 and C4 were grouped as low (<0.9 g/L and <0.2 g/L), moderate (0.9-1.8 g/L and 0.2–0.3 g/L), and high (>1.8 g/L and >0.03 g/L), respectively, which were empirically derived from the HCs distribution.

The serum IL-17A assay was performed according to the guidelines of the the manufacturers of human IL-17A ELISA Kit in pg/mL (intraassay: coefficients of variation (CV)<8%, inter-assay: CV <10%, MyBioSource, United States). The detected level of IL-17A was scaled as low (<25 pg/mL), moderate (25-50 pg/mL) and high (>50 pg/mL), which was empirically derived from the HCs distribution.

All immunodiagnostic assays were performed in the same laboratory following a uniform protocol for all subjects, with duplicate measurements. Laboratory staff were blinded to group allocation. Information regarding CV intra- and inter-assay was not available for all assays.

Statistical analysis

SPSS version 21 was utilized to perform the analysis of results (IBM, Armonk, NY, USA). The study included continuous variables (IL-17A, C3, C4, APL-IgM, APL-IgG) for which all have been checked for normality by visual inspection of histograms and the Shapiro-Wilk test. Inferential statistical tests performed were based on whether the continuous variable was normally distributed or not. For normally distributed variables, independent sample t-tests were used to compare two independent groups (SLE patients and HC). Categorical variables were analyzed using chi-square tests, with frequency distributions generated from descriptive statistics (mean, standard deviations (SD), frequency, percentage) for both demographic characteristics of

study participants and biomarker profiles among study groups. To identify the correlation between biomarker variables, the correlation coefficients were calculated (using either Pearson correlation coefficients for normally distributed variables or Spearman correlation coefficients for nonnormally distributed variables). Statistical significance was assessed using a cutoff value of p-value <0.05 for statistical significance.

Results

Demographic and clinical characteristics

Table 1 shows that the mean age of SLE patients (31.7± 9.6 years) and HC (31.9±9.4 years) was nearly identical, indicating a proper age matching. Females predominated in both groups, especially among patients with SLE (96.4%), consistent with the known female predominance in the disease. The mean duration of the disease in SLE patients was 3.25±2.77 years, including both newly diagnosed and treated cases. More than half of the patients (54.5%) reported a positive family history of SLE, highlighting a genetic predisposition. Most of the participants lived in urban settings with a marginally higher proportion of patients (63.6%), which can be attributed to environmental or access to care factors. Most of patients (74.5%) were in treatment, while 25.5% were newly diagnosed. Overall, the groups were well matched in age, sex and residence, supporting the validity of subsequent comparisons.

Table 1. Basic characteristics of the studied groups

Parameters/Study groups		SLE (n=110)	HCs (n=70)	Total (n=180)
Age (Year) (Mean±SD)		31.7±9.6	31.9±9.4	31.8±9.5
Disease duration (Year) (Mean±SD)		3.25±2.77	0±0	–
Sex (Frequency,%)	Males	4 (3.6)	6 (8.6)	10 (5.6)
	Females	106 (96.4)	64 (91.4)	170 (94.4)
SLE history (Frequency,%)	Present	50 (54.5)	0 (0)	50 (27.8)
	Absent	60 (45.5)	70 (100)	130 (72.2)
Residence (Frequency,%)	Urban	70 (63.6)	40 (57.1)	110 (61.1)
	Rural	40 (36.4)	30 (42.9)	70 (38.9)
Treatment status (Frequency,%)	On treatment	82 (74.5)	–	–
	Newly diagnosed	28 (25.5)	–	–

ANA positivity

The positive frequency was statistically elevated (p<0.05) in subjects with SLE (100%) than in the HCs group (5.7%) (Table 2).

Table 2. Distribution of study groups according to ANA*

Study groups	ANA				p
	Positive		Negative		
	Frequency	Percent	Frequency	Percent	
SLE (n=110)	110	100	0	0	
HCs (n=70)	4	5.7	66	94.3	SLE × HC: <0.05
Total (n=180)	92	51.1	88	48.9	

* x – indicate comparison

Serum IL-17A Levels

Table 3 shows that the vast majority of subjects with SLE had elevated levels of serum IL-17A (83.6%) compared to HC (17.1%) with a statistical difference (p<0.05). Regarding the mean IL-17A titers (Table 3), the highest was in the SLE group (516.2±421.5 pg/mL) compared to the HCs (31.9±19.3 pg/mL) with statistical significance (p<0.05).

Table 3. Distribution of study groups according to human IL-17A*

Study groups	Human IL-17A (pg/mL)				Mean±SD	p
	Low	Moderate	High	Total		
	FR (%)	FR (%)	FR (%)	FR (%)		
SLE (n=110)	2 (1.8)	16 (14.6)	92 (83.6)	110 (100)	516.2± 421.5	SLE x HC: <0.05
HCs (n=70)	31 (44.3)	27 (38.6)	12 (17.1)	70 (100)	31.9±19.3	
Total (n=180)	33 (18.3)	43 (23.9)	104 (57.8)	180 (100)	327.8±289.8	

* x – comparison, low – <25 pg/mL, moderate – 25–50 pg/mL, high – >50 pg/mL, FR –frequency

Complement components

The frequency of low C4 level was significantly elevated (p<0.05) among the members of the SLE disease (49.1%) than among subjects with HC (17.1%). Table 4 also showed the mean titers of C4 which exhibited a significantly (P<0.05) decreased level among subjects of the SLE group subjects (0.25±0.21 g/L) compared to an elevated level among subjects of the HC group (0.28±0.08 g/L). Regarding serum C3 in this study, most of the SLE subjects (61.8%) were at a low level (Table 4) compared to the HC (0%) with a statistical significance (p<0.05). The mean titer for C3 was lowest in the SLE group (0.92±0.56 g/L) than in subjects (1.22±0.14 g/L) with valuable differences (p<0.05) (Table 4).

Table 4. Distribution of study groups according to complement C4 and C3*

Study groups	Complement C4 (g/L)				Mean±SD	p
	Low	Moderate	High	Total		
	FR(%)	FR(%)	FR(%)	FR(%)		
SLE (n=110)	54 (49.1)	13 (11.8)	43 (39.1)	110 (100)	0.25±0.21	SLE x HC: <0.05
HCs (n=70)	12 (17.1)	32 (45.7)	26 (37.2)	70(100)	0.28±0.08	
Total (n=180)	66 (36.7)	45 (25)	69 (38.3)	180 (100)	0.26±0.17	
Study groups	Complement C3 (g/L)				Mean±SD	p
	Low	Moderate	High	Total		
	FR (%)	FR (%)	FR (%)	FR (%)		
SLE (n=110)	68 (61.8)	33 (30)	9 (8.2)	110 (100)	0.92±0.56	SLE x HC: <0.05
HCs (n=70)	0 (0)	70 (100)	0 (0)	70 (100)	1.22±0.14	
Total (n=180)	68 (37.8)	103 (57.2)	9 (5)	180 (100)	1.04±0.47	

* x – comparison, low C4 – <0.2 g/L, moderate C4 – 0.2–0.3 g/L, high C4 – >0.3 g/L, low C3 – <0.9 g/L, moderate C3 – 0.9–1.8 g/L, high C3 – >1.8 g/L, FR – frequency

Antiphospholipid antibodies

The frequency percentage of positive level serum APL-IgM and APL-IgG was significantly (p<0.05) elevated

in the SLE subjects 68/110 (61.8%) and 57/110 (52%), respectively, compared to the HCs 0/70 (0% for both auto-Abs) group. For the mean titer, the SLE group exhibited the highest mean titer (12.28±4.02 U/mL and 10.89±3.74 U/mL, respectively) compared to the HCs group (5.67±0.98 U/mL and 4.30±0.74, respectively) with statistical differences (p<0.05).

Table 5. Distribution of study groups according to IgM/IgG APL Abs*

Study groups	APL-IgM (U/mL)			Mean±SD	p
	Positive	Negative	Total		
	FR (%)	FR (%)	FR (%)		
SLE (n=110)	68 (61.8)	42 (38.2)	110 (100)	12.28±4.02	
HCs (n=70)	0 (0)	70 (100)	70 (100)	5.67±0.98	SLE x HC: <0.05
Total (n=180)	68 (37.8)	112 (62.2)	180 (100)	9.71±4.54	
Study groups	**APL-IgG (U/mL)**			**Mean±SD**	**p**
	Positive	**Negative**	**Total**		
	FR (%)	**FR (%)**	**FR (%)**		
SLE (n=110)	57 (52)	53 (48)	110 (100)	10.89±3.74	
HCs (n=70)	0 (0)	70 (100)	70 (100)	4.30±0.74	SLE x HC: <0.05
Total (n=180)	57 (32)	123 (68)	180 (100)	8.33±4.37	

* x – comparison, FR – frequency

Correlation between ANA and other biomarkers

In SLE and HC subjects with a positive ANA, the mean levels of IL-17A were significantly higher (56.1 pg/mL in SLE vs 49.3 pg/mL for HC) than those of HCs subjects who had negative ANA (30.8 pg/mL). In the SLE cohort with positive ANA, the C3 and C4 were significantly lower (0.92 g/L C3 and 0.25 g/L C4) than those of the subjects with negative ANA HC (1.22 g/L C3 and 0.27 g/L C4). The mean concentration of serum APL-IgM and APL-IgG was highest for positive ANA (12.2 U/mL IgM and 10.8 U/mL IgG) compared to negative individuals with ANA HC (5.6 U/mL IgM and 4.2 U/mL IgG) with statistical significance (p<0.05) (Table 6).

Table 6. The correlation between ANA and other study biomarkers*

Biomarkers	ANA				p
	SLE	HCs			
	Ve^+ (n=110)	Ve^+ (n=4)	Ve^- (n=66)	T (n=70)	
IL-17A (pg/mL), mean	516.1	49.3	30.8	31.9	<0.05
C3 (g/L), mean	0.92	1.1	1.22	1.22	<0.05
C4 (g/L), mean	0.25	0.28	0.27	0.28	<0.05
IgM APL Ab (U/mL), mean	12.2	6.8	5.6	5.6	<0.05
IgG APL Ab (U/mL), mean	10.8	4.9	4.2	4.3	<0.05

* Ve^+ – positive, Ve^- – negative, T – total

Correlation between APL and other biomarkers

The difference in the mean IL-17A titer between subjects with positive, and negative serum levels of serum APL-IgG and APL-IgM was not significant (p>0.05). SLE subjects with positive levels of APL-IgG and APL-IgM exhibited the lowest mean titer (0.61 g/L and 0.65 g/L, respectively) of serum C3, compared to SLE subjects with negative APL-IgG and APL-IgM (1.2 g/L and 1.3 g/L, respectively) with valuable differences (p<0.05). The findings of the Pearson's correlation reported that there was an inverse relationship between APL-IgG, APL-IgM, and C3 levels in the SLE group (r=-0.466 for IgG and r=-0.535 for IgM). For the mean C4 titer, the level was statistically (p<0.05) lower in subjects with positive levels of APL-IgG and APL-IgM (0.17 g/L and 0.19 g/L, respectively) than negative levels of APL-IgG and APL-IgM (0.33 g/L for both). The statistical Pearson correlation showed that the concentration of APL-IgG and APL-IgM was correlated with the level of C4 (r=-0.303 and r=-0.298, respectively) (Table 7).

Table 7. Correlation between APL Abs and other study biomarkers*

Biomarkers	APL Abs positivity in SLE group						Spearman's ρ	p
	IgG APL Abs			IgM APL Abs				
	Ve^+ (n=57)	Ve^- (n=53)	T (n=110)	Ve^+ (n=68)	Ve^- (n=42)	T (n=110)		
IL-17A (pg/mL), mean	527.5	504	516.2	544.1	470.9	516.2	IgG: -0.057 IgM: 0.029	>0.05
C3 (g/L), mean	0.61	1.2	0.92	0.65	1.3	0.92	IgG: -0.466 IgM: -0.535	<0.05
C4 (g/L), mean	0.17	0.33	0.25	0.19	0.33	0.25	IgG: -0.303 IgM: -0.298	<0.05

* Ve^+ – positive, Ve^- – negative, T – total

Correlation between IL-17A and complement components

There was no valuable correlation between the level of IL-17A and the concentrations of C3 or C4 in both study groups.

Table 8. The correlation between IL-17A and complements components*

Biomarkers	IL-17A (pg/mL)								Spearman's ρ	p
	SLE				HCs					
	L (n=2)	M (n=16)	H (n=92)	T (n=110)	L (n=31)	M (n=27)	H (n=12)	T (n=70)		
C3 (g/L), mean	0.97	0.86	0.93	0.92	1.22	1.2	1.2	1.2	HC: -0.028 SLE: -0.131	>0.05
C4 (g/L), mean	0.13	0.28	0.24	0.25	0.28	0.27	0.27	0.28	HC:-0.167 SLE: -0.085	>0.05

* T – total, L – low (<25 pg/mL), M – moderate (25–50 pg / mL), H – high (>50 pg/mL), FR – frequency

Discussion

The present study demonstrated significantly higher levels of ANA, IL-17A and APL-Abs (IgM and IgG) in patients with SLE compared with HCs, accompanied by markedly reduced C3 and C4 concentrations. Positive correlations between ANA and both IL-17A and APL-

Ab suggest potential immunological interaction among these factors in SLE pathogenesis. On the contrary, the negative correlations observed between ANA or APL-Abs and C3/C4 indicate continued activation of the immune complex and complement consumption characteristic of active disease. Although IL-17A was not significantly correlated with C3 or C4, its elevation suggests further evidence of immune dysregulation. Collectively, these findings substantiate the role of auto-Abs, cytokine imbalance, and complement depletion in SLE activity without implying causation.

A key outcome of this study was the significantly increased positivity for ANA in the SLE group in comparison to the HCs (Table 2). This finding is consistent with previously published research documenting ANA positivity in 92.3% of SLE patients.[14] Furthermore, serum IL-17A was also detected to be significantly higher in SLE subjects compared to controls (Table 3), which confirms similarly published studies that observed increased expression of IL-17 in autoimmune disorders.[15–17] The findings support the proposal that ANA and IL-17A being both associated with immunopathological abnormalities related to SLE, possibly contributing to inflammatory mechanisms in SLE.

The study found that SLE participants had significantly lower serum C3 and C4 levels than HC (Table 4). These results corroborate results from previous research conducted by Trouw et al.,[18] Aringer et al.,[13] and others,[19,20] who also found evidence of hypocomplementemia in SLE. The decreased levels of C3 and [C4] likely reflect complement activation and consumption with immune complexes, a well-established feature in the pathophysiology of SLE. The reproducibility of similar results in the literature suggests that C3 and C4 levels may have value when determining the contributions of the complement system to symptoms seen in SLE. Although the results may help determine disease activity or changes in treatment response, they should never be used as definitive markers of disease progression,[10] but rather in conjunction with other clinical and laboratory markers.

As shown in Table 5, serum APL-IgM concentrations were significantly higher in subjects with SLE than in HCs. This is consistent with previous studies that demonstrate that SLE cases tend to exhibit higher APL Abs, including the IgM isotype, and an association with thrombotic risk and related clinical features.[21,22] Similarly, we found that higher APL-IgM concentrations and incidence in SLE people suggest that this is relevant to disease characterization but that we are not making claims about causation. APL-IgG concentrations were also higher in measured SLE subjects, again significant compared to HC (Table 5). This is consistent with recent work by Farina et al.[23] who similarly found increased APL-IgG levels in their SLE cases. Our current study did not replicate the claims of APL-IgG positivity rates in the work of Ünlü et al.[24], who reported lower frequencies among subjects with SLE compared to controls. As before, this can be largely attributed to population characteristics, diagnostic reference, or the variability of the detection protocol. In summary, APL-IgG and APL-IgM concentrations were elevated over HCs and the patterns of their association in SLE support the previous investigation, while at the same time indicating the threshold difference attributable to variability in the detection or characteristics of the study population.

Beringer et al.[25] demonstrated that IL-17A is involved in immune responses and dysregulation of IL-17A has been associated with autoimmune diseases. Similarly, in the current study, we observed increased levels of IL-17A in participants who tested positive for ANA. Using both study groups, we found evidence that indicates a positive correlation between IL-17A and ANA (Table 6). These findings are consistent with previous reports showing a link between IL-17A and inflammation and auto-Abs production in SLE. In particular, Ebrahimi et al.[26] demonstrated that higher concentrations of IL-17A were related to increased disease activity in SLE. The current study observed positive correlations between IL-17A positivity and ANA positivity.

Furthermore, there was an inverse correlation between ANA and C3 and C4 in both the SLE and HC groups. The presence of decreased complement levels may indicate the formation/activation of immune complexes, in agreement with prior studies that have found decreased levels of C3 and C4 in ANA-positive SLE patients.[27-29] These results indicate that the measurement of complement depletion may indicate the activation of the immune system rather than a definitive measure of disease activity. In healthy individuals, the weaker effects of ANA on complement levels may indicate subclinical immune activity or predisposition to autoimmunity.

Previous investigations[11] indicated that ANA-positive individuals, who do not demonstrate clinical evidence of autoimmune disease, may have minor alterations in complement, suggesting early immune dysregulation. Consistent with these results, we report evidence of positivity for ANA related to lower C3/C4 levels consistent with history of autoimmune disease, especially SLE. Overall, the evidence supports the notion that ANA is a marker of underlying immune alterations and may not serve as a discriminatory prognostic biomarker, but rather as an aid in evaluating disease activity.

The results of this investigation offer important information on the relationship between ANA and APL-Abs in SLE patients. SLE subjects who were ANA positive had significantly higher levels of APL-IgM and APL-IgG concentrations (Table 6), suggesting a relationship between ANA positivity and higher levels of APL-Abs. This finding is consistent with earlier stud-

ies demonstrating that the coexistence of APL-Ab with ANA can be associated with increased disease activity and an increased risk of thrombotic events in SLE.[30] The observation that APL-Ab may be associated with ANA-positivity indicates an increased risk of thrombotic complications and pregnancy outcomes associated with APS.[31] These findings reinforce the idea that ANA and APL-Abs may have similar immunopathogenesis linked to endothelial injury and subsequent thrombo-inflammatory events.[32]

Table 7 indicates that there were no significant differences in IL-17A levels among SLE patients with positive serum APL-IgG and APL-IgM Abs, indicating that IL-17A is generally not associated with APL-Ab activity among SLE patients. Furthermore, Doreau et al.[33] found that SLE patients had increased IL-17A without association with APL-Abs, suggesting that IL-17A could contribute to the presence of SLE pathology relative to APL-Abs or IgM or IgG levels. Another study[34] suggested that while IL-17A may be indicative of overall disease activity, it does not correlate with the presence of APL-Abs.

These findings are consistent with the idea that IL-17A acts as part of a complex cytokine network in SLE and that it may not be clearly related to APL-Abs. The results indicated a statistically significant negative correlation between APL (APL-IgG and APL-IgM) and C3 and C4 in SLE patients (Table 7). Previous investigations[35] reported that the presence of APL Abs was associated with lower complement levels, which, in a similar fashion, suggested that these Abs may lead to complement consumption and hypocomplementemia in SLE. Overall, these findings may begin to support the potential to monitor these APL-Ab along with complement components as a possible way to monitor disease activity and help identify individuals at risk of worse outcomes who may need closer follow-up, as indicated by Fanouriakis et al.[36]

The absence of a meaningful correlation between IL-17A concentrations and C3 or C4 levels in both the SLE and HC cohorts (Table 8) indicates that IL-17A can contribute to the pathogenesis of SLE independent of complement components. These results corroborate the reported findings[35] showing that, although SLE patients have higher levels of IL-17A, there are no explicit correlations with complement activation, which also suggests independent pathogenic mechanisms. IL-17A has the ability to amplify an inflammatory response, although the role of this cytokine in triggering complement activation may be through an indirect pathway, and possibly unknown context-dependent pathways, through local inflammatory mediators and/or interactions with other cytokines.[10] However, the IL-17A signaling pathway and complement systems of complement will likely have several overlaps to consider during all aspects of the immune response and may be appropriate for additional investigation.

IL-17A levels showed a marked increase in patients with SLE and these levels correlated with ANA in the circulation but showed no association with C3 and C4, suggesting a possible independent role in the dysregulation of the immune response. IL-17A is an interesting and unvalidated biomarker in SLE that has not been extensively studied enough to confirm if it is clinically relevant for disease monitoring, patient stratification, or as a potential therapeutic target.

This study adds new information because we added an analysis of how the biomarkers in the current study interact with each other in the same group of patients. From correlation analysis, we found a strong positive correlation between ANA and IL-17A and an inverse correlation between APL Abs and complement levels. These findings have not previously been extensively published in Middle Eastern populations. These findings provide new information on how classical serological markers work together in a specific immunogenetic context and they provide evidence for possible population-specific immune dysregulation pathways in SLE.

Study limitations

The study does not report a disease activity score, but still identifies clear differences in immunology between patients diagnosed with SLE versus reportedly HC. Although limited to demonstrating the cross-sectional nature of these data, the results represent a snapshot of differences in biomarker profiles. The selection of ANA, IL-17A, C3, C4, and APL-Abs represents critical pathways associated with SLE and lends scientific relevance to the findings of the study. Although the sample size of the HC group was lower than that of the SLE group, the subjects were selected to be comparable, thus establishing internal validity for this study. While the correlations between these two groups were relatively weak, the statistically significant and biologically relevant findings of these correlations lend support to the conclusions drawn in this study. The restricted biomarker panel used in this study may limit the identification of additional contributing pathways, but provides greater specificity for evaluating the central mechanisms that lead to the pathology of SLE. Therefore, despite these limitations, the study provides compelling evidence for an underlying immunologic dysregulation in patients with SLE. This study consisted of only Iraqi patients and did not include participants from other countries or ethnic backgrounds. Furthermore, no detailed environmental data were collected as this was not a primary goal of the study.

Conclusion

According to observations, SLE is marked by a significant increase in positive ANA and IL-17A levels, and a significant decrease in C3 and C4 levels, compared to HCs. Since there is a positive correlation between ANA and IL-17A, the involvement of both substances in the

pathophysiology of SLE can be speculated. The higher levels of APL-IgM and APL-IgG observed in SLE compare to HCs, which were inversely correlated to C3 and C4, revealed the complicated relationships between auto-Abs and complement proteins in SLE, as well. Collectively, these biomarkers indicate significant immunological dysregulation in SLE. No direct link was reported between IL-17A levels and C3 or C4.

Acknowledgments

The authors thank Southern Technical University, Nasiriyah Technical Institute, for their support and facilities. Gratitude is also extended to the staff of the Imam Al-Hussein Teaching Hospital and all participants who contributed to this study.

Declarations

Funding
Self-fund.

Author contributions
Conceptualization, K.H.A.A.; Methodology, L.S.F.; Software, L.S.F.; Validation, K.H.A.A; Formal Analysis, L.S.F.; Investigation, K.H.A.A.; Resources, L.S.F.; Data Curation, K.H.A.A.; Writing – Original Draft Preparation, K.H.A.A.; Writing – Review & Editing, L.S.F.; Visualization, K.H.A.A.; Supervision, L.S.F.; Project Administration, K.H.A.A.; Funding Acquisition, L.S.F.

Conflicts of interest
The author declared no conflicts of interest.

Data availability
The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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