

Serum Interferon- γ as a Biomarker of Cardiovascular Complications in Systemic Lupus Erythematosus

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ABSTRACT

Background: Systemic lupus erythematosus (SLE) is associated with an elevated risk of premature cardiovascular disease (CVD). Traditional serological markers and standard lipid indices often demonstrate limited utility in stratifying this macrovascular risk. **Purpose:** This study evaluates the potential of the Th1-cytokine Interferon- γ (IFN- γ) to discriminate concurrent CVD complications in patients with SLE and explores its cardiometabolic associations. **Methods:** A case-control study was conducted with 120 age- and gender-matched participants, divided equally into three groups (n = 40 per group): SLE with CVD, SLE without CVD, and healthy controls. Serum concentrations of IFN- γ , C-reactive protein (CRP), anti-dsDNA autoantibodies, and lipid profiles were quantified. Statistical and Receiver Operating Characteristic (ROC) curve analyses were utilized to evaluate diagnostic potential and inter-variable associations. **Results:** Both CRP and anti-dsDNA levels were significantly elevated in the two SLE cohorts compared to controls (p = 0.001); however, they did not significantly differentiate between SLE patients with and without concurrent CVD. Standard lipid fractions showed no significant variation across the three groups. In contrast, systemic IFN- γ levels were significantly higher in the CVD-complicated SLE cohort (218.17 ± 21.9 pg/mL) compared to the uncomplicated SLE group (196.06 ± 19.5 pg/mL) (p = 0.001). ROC analysis indicated a strong discriminative capacity for IFN- γ in identifying SLE-CVD (AUC = 0.973; sensitivity 95.0%, specificity 97.5% at a cutoff > 162.29 pg/mL). Bivariate analysis demonstrated a positive correlation between IFN- γ and total cholesterol specifically within the CVD-complicated subgroup (r = 0.336, p = 0.034). **Conclusion:** IFN- γ shows strong potential as a discriminative biomarker for concurrent cardiovascular complications in SLE, demonstrating better stratification than conventional inflammatory and lipid markers within this study cohort. Its correlation with total cholesterol in the CVD phenotype suggests a possible Th1-mediated cardiometabolic link that warrants further longitudinal investigation.

Keywords: Systemic Lupus Erythematosus; Cardiovascular Disease; Interferon-gamma; Biomarker; Atherosclerosis; Autoimmunity.

Article Information

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INTRODUCTION

Systemic lupus erythematosus (SLE) is widely characterized as a heterogeneous, systemic autoimmune disorder that may involve multiple organ systems through a complex interplay of immune dysregulation and autoantibody production (1,2). While advancements in immunomodulatory therapies

have substantially improved overall survival rates, premature cardiovascular disease (CVD) has concurrently emerged as a primary cause of morbidity and mortality within this patient population (3). Notably, the accelerated atherogenesis observed in SLE is often not fully explained by traditional Framingham risk factors alone, suggesting that intrinsic, autoimmune-driven inflammatory pathways

may significantly contribute to vascular pathogenesis (4,5).

In routine clinical practice, non-specific acute-phase reactants, such as C-reactive protein (CRP), alongside conventional lipid panels, are frequently utilized to monitor baseline inflammatory and metabolic states. However, recent literature indicates that these standard parameters may lack the necessary sensitivity and specificity to accurately stratify macrovascular risk or reliably differentiate between active autoimmune flares and concomitant cardiovascular complications (6,7). Furthermore, established pro-inflammatory cytokines, particularly TNF- α and IL-6, play well-documented roles in driving the basal systemic inflammation, B-cell hyperactivity, and subsequent autoantibody production characteristic of SLE. However, while these markers strongly correlate with general disease flares, their specific predictive value and sensitivity for stratifying accelerated atherogenesis and macrovascular complications remain challenging, necessitating the exploration of alternative immune axes (8). Consequently, the identification of highly discriminative serological biomarkers that can precisely reflect the specific atherogenic burden in SLE remains an ongoing imperative in clinical immunology. Accumulating evidence suggests a pivotal role for cytokine-mediated endothelial dysfunction, with particular emphasis on the T-helper 1 (Th1) inflammatory axis (9).

Interferon-gamma (IFN- γ), a central Th1-signature cytokine, is currently hypothesized to be a key orchestrator in promoting vascular inflammation, macrophage activation, and localized lipid oxidation, processes intimately linked to plaque formation and destabilization (10,11). Therefore, this study uniquely evaluates the discriminative capacity of systemic IFN- γ for macrovascular complications in SLE, exploring a novel

immune-metabolic interplay that distinguishes it from traditional inflammatory markers.

METHODS

Study Design and Population

This research was structured as a case-control study, conducted at the Rheumatology and Cardiology departments of Baghdad Teaching Hospital, Medical City Complex, located in Baghdad, Iraq, between November 2025 and February 2026. The study enrolled a total of 120 adult participants, systematically stratified into three equal cohorts (n = 40 per group): patients diagnosed with SLE complicated by CVD, SLE patients without CVD, and a healthy control group matched for age and gender. To ensure robust statistical validity and minimize Type II errors, the specimen size was determined *a priori* using standard power analysis protocols; anticipating a medium effect size based on prior immunological literature, the cohort of 120 subjects was calculated to yield a statistical power exceeding 90% at an alpha significance level of 0.05. The ethical integrity of this study was formally validated, operating under the full ethical approval granted by the Institutional Review Board (IRB) of the Faculty of Medicine, University of Kufa (Approval Protocol No. 69693). Written informed consent was obtained from all participants prior to enrollment, in strict adherence to the principles outlined in the Declaration of Helsinki.

Inclusion and Exclusion Criteria

Inclusion in the patient cohorts required a confirmed diagnosis of SLE, established by specialized consultant rheumatologists, fulfilling the updated American College of Rheumatology (ACR) or Systemic Lupus International Collaborating Clinics (SLICC) classification criteria. Patients in the CVD-complicated cohort were required to have documented evidence of macrovascular involvement (e.g., ischemic heart disease, myocardial infarction, or distinct atherosclerotic

plaque formation) confirmed by expert cardiological assessment and relevant imaging modalities. Healthy controls were recruited from the general population, presenting with no personal or familial history of autoimmune or cardiovascular pathologies. To minimize confounding immunological and metabolic variables, stringent exclusion criteria were applied across all groups; individuals were excluded if they presented with active acute infections, concurrent malignancies, pregnancy, chronic kidney disease, other overlapping autoimmune disorders (such as rheumatoid arthritis), or if they had undergone recent significant alterations in high-dose immunosuppressive or lipid-lowering therapeutic regimens.

Specimen Collection and Preparation

Following a mandatory overnight fasting period of 10 to 12 hours, approximately 5 mL of peripheral venous blood was aseptically collected from each participant into plain, endotoxin-free gel-separator tubes. The whole blood specimens were allowed to clot naturally at room temperature for 30 minutes. Subsequently, the specimens were subjected to centrifugation at 3000 revolutions per minute (rpm) for 15 minutes to optimally separate the serum fraction. The extracted serum was meticulously aliquoted into sterile Eppendorf tubes to avoid repeated freeze-thaw degradation and immediately preserved at -80°C until the scheduled biochemical and immunological assays were simultaneously performed.

Biochemical Analysis

To evaluate the basal metabolic and acute-phase inflammatory state of the participants, comprehensive biochemical profiling was executed. The serum lipid panel, encompassing total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL), was quantified using standard enzymatic colorimetric assays on fully automated clinical chemistry analyzer (Cobas c 311 analyzer, Roche Diagnostics, Mannheim,

Germany). Concurrently, systemic concentrations of the acute-phase reactant CRP were measured to assess the general non-specific inflammatory burden utilizing a standardized particle-enhanced immunoturbidimetric assay on the same automated clinical chemistry analyzer (Cobas c 311, Roche Diagnostics).

Assessment of SLE Diagnostic Test

To ascertain the baseline autoimmune severity and confirm the pathological autoantibody profile inherent to SLE, circulating levels of anti-double-stranded DNA (anti-dsDNA) antibodies were quantitatively evaluated. This diagnostic parameter was measured utilizing a standardized, commercially available quantitative enzyme-linked immunosorbent assay (ELISA) kit (ELK Biotechnology; Catalogue No. ELK9142; Analytical Sensitivity: 0.099 ng/mL), routinely employed in clinical laboratory settings, serving as a critical serological index for disease activity and specific autoimmune dysregulation.

Measurement of Serum Cytokine (IFN-gamma)

The systemic quantification of the Th1-signature cytokine, Interferon-gamma (IFN-gamma), was executed utilizing a highly sensitive, specific quantitative sandwich Enzyme-Linked Immunosorbent Assay (ELISA). The analysis was strictly performed using a commercial kit manufactured by Bioassay Technology Laboratory (BT LAB) (China; Cat. No. E0105Hu; Analytical Sensitivity: 0.49 ng/ml). All analytical procedures, including reagent preparation, incubation intervals, and washing steps, were conducted in rigorous accordance with the manufacturer's standardized protocols. The final optical density (OD) of the immunochemical reaction was spectrophotometrically determined at a wavelength of 450 nm using a microplate reader, with absolute cytokine concentrations

interpolated from a standard curve generated concurrently (**Figure 1**).

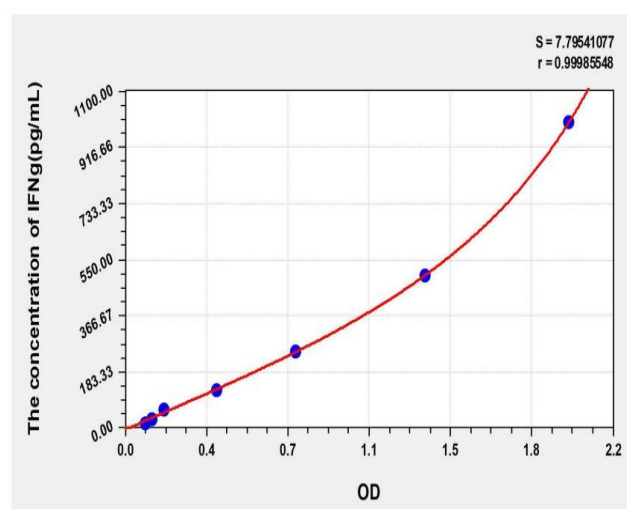


Figure (1): Human IFN- γ (Interferon

Statistical Analysis

All quantitative datasets were compiled and statistically analyzed utilizing the Statistical Package for the Social Sciences (SPSS) software (IBM Corp., Armonk, NY, USA). The normality of continuous data distribution was initially verified. Parametric data were expressed as Mean $\pm$ Standard Deviation (SD) or Standard Error (SE) and analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test for multiple group comparisons, or independent specimens t-tests for bi-group analyses. Non-parametric variables were evaluated using the Kruskal-Wallis test

with subsequent post-hoc evaluations. Bivariate correlational analyses were performed employing Pearson's or Spearman's rank coefficients, contingent upon data distribution. To assess the diagnostic capacity and optimal discriminative threshold of IFN- γ , Receiver Operating Characteristic (ROC) curves were constructed, and the area under the curve (AUC), sensitivity, and specificity were calculated, with optimal cutoffs determined via Youden's index. Across all analytical models, an alpha level of $p < 0.05$ was adopted to denote statistical significance.

RESULTS

Table 1 shows that the study cohort comprised 120 age- and gender-matched subjects distributed equally among SLE patients with CVD, SLE patients without CVD, and healthy controls, with no significant inter-group variance in baseline age ($p = 0.991$) or gender distribution ($p = 0.410$). However, both SLE cohorts exhibited markedly elevated BMI ($p = 0.029$) and body weight ($p = 0.041$) compared to healthy controls, with highly significant differences in obesity classification across groups ($p < 0.001$). A positive family history of SLE was significantly more prevalent in patients without CVD (37.5%) than in those with CVD (15.0%) ($p = 0.022$), whereas disease duration did not significantly differ between the two patient cohorts ($p = 0.822$).

Table 1. Baseline demographic and clinical characteristics of the study population stratified by SLE and CVD status.

Characteristics	SLE with CVD (n=40)	SLE without CVD (n=40)	Healthy Control (n=40)	P-value
Gender				
Male	5 (12.5%)	4 (10.0%)	8 (20.0%)	0.410 NS
Female	35 (87.5%)	36 (90.0%)	32 (80.0%)	
Age (years)				
Mean $\pm$ SD	36.73 $\pm$ 9.35	36.70 $\pm$ 9.56	36.50 $\pm$ 8.82	0.991 NS
Range	16 – 65	16 – 63	20 – 65	
BMI (kg/m²)				

Characteristics	SLE with CVD (n=40)	SLE without CVD (n=40)	Healthy Control (n=40)	P-value
Mean $\pm$ SD	27.88 $\pm$ 5.32 ^A	29.19 $\pm$ 6.84 ^A	24.61 $\pm$ 2.04 ^B	0.029 S
Weight (kg)				
Mean $\pm$ SD	74.55 $\pm$ 9.69 ^A	78.57 $\pm$ 10.2 ^A	66.61 $\pm$ 8.77 ^B	0.041 S
Height (cm)				
Mean $\pm$ SD	163.46 $\pm$ 8.94	164.59 $\pm$ 7.62	164.20 $\pm$ 8.56	0.845 NS
Obesity Classification				
Normal weight	17 (42.5%)	13 (32.5%)	28 (70.0%)	< 0.001 S
Overweight	12 (30.0%)	11 (27.5%)	12 (30.0%)	
Obese	11 (27.5%)	16 (40.0%)	0 (0%)	
Family History of SLE				
Positive	6 (15.0%)	15 (37.5%)	—	0.022 S
Negative	34 (85.0%)	25 (62.5%)	—	
Duration of Disease				
< 5 years	23 (57.5%)	22 (55.0%)	—	0.822 NS
$\geq$ 5 years	17 (42.5%)	18 (45.0%)	—	

Data: Mean $\pm$ SD or n (%). Continuous variables compared by one-way ANOVA; categorical variables by Chi-square (χ^2) or Fisher's exact test. ^A, ^B: groups sharing a superscript do not differ significantly by post-hoc analysis; groups with different superscripts differ significantly ($p < 0.05$). S = Significant ($p < 0.05$); NS = Not Significant. Family history and disease duration apply to SLE groups only.

Table 2 presents the biochemical and immunological profiles across the three study groups. Anti-dsDNA autoantibody titers showed a highly significant global variance ($p = 0.001$); both CVD-complicated SLE (2.84 ± 0.63 IU/mL) and uncomplicated SLE (3.13 ± 0.85 IU/mL) cohorts exhibited significantly elevated concentrations relative to healthy controls (2.23 ± 0.57 IU/mL), while no significant quantitative difference was observed between the two SLE subgroups ($p > 0.05$). CRP quantification similarly demonstrated highly significant global variance ($p = 0.001$), with profoundly elevated levels in CVD-complicated SLE (13.10 ± 2.27 mg/L) and uncomplicated SLE (12.25 ± 2.09 mg/L) compared to healthy

controls (2.21 ± 0.46 mg/L), and no significant difference between the two SLE cohorts ($p > 0.05$). Analysis of IFN- γ revealed highly significant systemic up-regulation ($p = 0.001$), with post-hoc analysis delineating distinct profiles across all three groups: CVD-complicated SLE (218.17 ± 21.9 pg/mL) $>$ uncomplicated SLE (196.06 ± 19.5 pg/mL) $>$ healthy controls (107.21 ± 12.4 pg/mL). In contrast, the serum lipid profile showed no statistically significant variance across cohorts for total cholesterol ($p = 0.889$), triglycerides ($p = 0.541$), LDL ($p = 0.153$), or HDL ($p = 0.615$), indicating that gross lipemic indices do not differentiate the SLE clinical phenotypes based on macrovascular status.

Table 2. Biochemical and Immunological Profiles across Study Groups.

Parameters	SLE with CVD (n=40)	SLE without CVD (n=40)	Healthy Control (n=40)	P-value
Anti-dsDNA (IU/mL), Kruskal-Wallis test				
Mean $\pm$ SD	2.84 $\pm$ 0.63 ^A	3.13 $\pm$ 0.85 ^A	2.23 $\pm$ 0.57 ^B	0.001**
Range	1.69 – 6.54	1.59 – 7.54	0.71 – 2.93	
CRP (mg/L), Kruskal-Wallis test				
Mean $\pm$ SE	13.10 $\pm$ 2.27 ^A	12.25 $\pm$ 2.09 ^A	2.21 $\pm$ 0.46 ^B	0.001**
Range	0.40 – 47.00	0.30 – 98.00	0.80 – 8.00	
IFN-γ (pg/mL), One-way ANOVA with post-hoc Tukey test				
Mean $\pm$ SD	218.17 $\pm$ 21.9 ^A	196.06 $\pm$ 19.5 ^B	107.21 $\pm$ 12.4 ^c	0.001**
Range	126.87 – 323.92	111.10 – 330.89	77.76 – 182.86	
Lipid Profile — Kruskal-Wallis test				
Cholesterol (mg/dL)				
Mean $\pm$ SE	102.45 $\pm$ 6.78 ^A	105.82 $\pm$ 5.99 ^A	107.50 $\pm$ 9.19 ^A	0.889 NS
Range	50 – 199	51 – 198	53 – 198	
Triglycerides (mg/dL)				
Mean $\pm$ SE	76.31 $\pm$ 6.71 ^A	68.95 $\pm$ 4.75 ^A	77.25 $\pm$ 8.17 ^A	0.541 NS
Range	21 – 147	19 – 131	20 – 137	
LDL (mg/dL)				
Mean $\pm$ SD	62.15 $\pm$ 6.06 ^A	64.50 $\pm$ 6.25 ^A	45.50 $\pm$ 5.91 ^A	0.153 NS
Range	3 – 115	10 – 123	10 – 99	
HDL (mg/dL)				
Mean $\pm$ SE	42.31 $\pm$ 2.76 ^A	43.33 $\pm$ 2.25 ^A	44.3 $\pm$ 2.95 ^A	0.615 NS
Range	33.8 – 58.9	33.6 – 60.1	35.6 – 59.9	

Data: Mean $\pm$ SD or Mean $\pm$ SE and range. Kruskal-Wallis test (Anti-dsDNA, CRP, Lipids); one-way ANOVA with post-hoc Tukey test (IFN- γ). ^A, ^B, ^c: groups bearing different superscripts differ significantly; groups sharing the same superscript do not differ. **: $p < 0.001$; NS = Not Significant ($p \geq 0.05$).

Table 3 presents subgroup analyses evaluating the influence of familial predisposition and disease chronicity on CRP, IFN- γ , and the lipid profile within both SLE subsets. Neither a positive family history of SLE nor prolonged disease duration (≥ 5 years) significantly modulated CRP concentrations, IFN- γ levels, or any lipid fraction in either cohort (all $p > 0.05$). Specifically, within the

CVD-complicated SLE group, CRP showed no significant variation attributable to family history ($p = 0.341$) or disease duration ($p = 0.281$), a pattern identically mirrored in the uncomplicated SLE subgroup for both family history ($p = 0.198$) and disease chronicity ($p = 0.576$). IFN- γ remained statistically uniform irrespective of familial inheritance ($p = 0.429$ and $p = 0.506$, respectively) or temporal

progression ($p = 0.109$ and $p = 0.902$, respectively). All lipid parameters likewise showed no significant stratification by either variable (all $p > 0.05$), establishing that the

inflammatory and lipid profiles in these phenotypes operate independently of these anamnestic and temporal parameters.

Table 3. Subgroup analysis of targeted parameters stratified by familial predisposition and disease chronicity.

Stratification	n (CVD / no CVD)	SLE with CVD Mean $\pm$ SD/SE	SLE without CVD Mean $\pm$ SD/SE
A. CRP (mg/L), Mean $\pm$ SE Independent samples t-test			
Family History of SLE			
Positive	6 / 15	14.92 $\pm$ 2.44	14.31 $\pm$ 3.31
Negative	34 / 25	12.81 $\pm$ 2.66	10.95 $\pm$ 3.14
P-value		0.341 NS	0.198 NS
Disease Duration			
< 5 years	23 / 22	11.80 $\pm$ 2.44	10.17 $\pm$ 2.41
$\geq$ 5 years	17 / 18	14.66 $\pm$ 2.51	12.58 $\pm$ 3.68
P-value		0.281 NS	0.576 NS
B. IFN-γ (pg/mL) — Mean $\pm$ SD Independent samples t-test			
Family History of SLE			
Positive	6 / 15	205.57 $\pm$ 11.99	224.09 $\pm$ 29.4
Negative	34 / 25	238.09 $\pm$ 28.4	206.91 $\pm$ 28.8
P-value		0.429 NS	0.506 NS
Disease Duration			
< 5 years	23 / 22	237.1 $\pm$ 22.3	213.21 $\pm$ 32.1
$\geq$ 5 years	17 / 18	214.3 $\pm$ 21.5	216.42 $\pm$ 29.8
P-value		0.109 NS	0.902 NS
C. Lipid Profile (mg/dL) — Mean $\pm$ SE Independent samples t-test			
Cholesterol (mg/dL)			
Family Hx: Positive	6 / 15	117.5 $\pm$ 9.22	118.5 $\pm$ 9.47
Family Hx: Negative	34 / 25	99.79 $\pm$ 7.04	95.41 $\pm$ 6.31
P-value		0.358 NS	0.054 NS
Duration: < 5 yrs	23 / 22	100.73 $\pm$ 7.76	113.54 $\pm$ 8.61
Duration: $\geq$ 5 yrs	17 / 18	104.76 $\pm$ 6.98	96.38 $\pm$ 6.87
P-value		0.774 NS	0.157 NS
Triglycerides (TG, mg/dL)			
Family Hx: Positive	6 / 15	80.21 $\pm$ 6.05	64.83 $\pm$ 6.95

Stratification	n (CVD / no CVD)	SLE with CVD Mean $\pm$ SD/SE	SLE without CVD Mean $\pm$ SD/SE
Family Hx: Negative	34 / 25	75.61 $\pm$ 6.19	72.31 $\pm$ 6.56
P-value		0.780 NS	0.441 NS
Duration: < 5 yrs	23 / 22	81.74 $\pm$ 7.46	76.72 $\pm$ 5.82
Duration: $\geq$ 5 yrs	17 / 18	68.94 $\pm$ 6.81	59.44 $\pm$ 7.38
P-value		0.274 NS	0.070 NS
LDL (mg/dL)			
Family Hx: Positive	6 / 15	53.16 $\pm$ 6.12	69.5 $\pm$ 6.31
Family Hx: Negative	34 / 25	63.73 $\pm$ 6.61	60.41 $\pm$ 7.73
P-value		0.152 NS	0.477 NS
Duration: < 5 yrs	23 / 22	63.88 $\pm$ 6.71	68.41 $\pm$ 6.31
Duration: $\geq$ 5 yrs	17 / 18	65.01 $\pm$ 5.92	59.72 $\pm$ 7.39
P-value		0.901 NS	0.497 NS
HDL (mg/dL)			
Family Hx: Positive	6 / 15	36.91 $\pm$ 2.17	43.76 $\pm$ 1.97
Family Hx: Negative	34 / 25	43.27 $\pm$ 2.31	42.97 $\pm$ 2.62
P-value		0.065 NS	0.757 NS
Duration: < 5 yrs	23 / 22	39.91 $\pm$ 1.98	42.84 $\pm$ 1.91
Duration: $\geq$ 5 yrs	17 / 18	45.98 $\pm$ 2.17	43.91 $\pm$ 2.13
P-value		0.124 NS	0.684 NS

Data: Mean $\pm$ SE (CRP, Lipids) or Mean $\pm$ SD (IFN- γ). n = sample size (SLE with CVD / SLE without CVD). Independent samples t-test. NS = Not Significant ($p \geq 0.05$).

Table 4 presents bivariate correlation analyses between serological and cardiometabolic parameters. For CRP, Spearman's rank correlation revealed a complete absence of significant linear association with anti-dsDNA titers in both CVD-complicated SLE ($r = 0.059$, $p = 0.717$) and uncomplicated SLE ($r = 0.249$, $p = 0.122$). For IFN- γ , Pearson's correlation revealed significant positive associations with anti-dsDNA titers in both CVD-complicated ($r = 0.326$, $p = 0.040$) and uncomplicated ($r = 0.373$, $p = 0.018$) SLE

cohorts, indicating a consistent coupling of Th1-cytokine activity with autoantibody production irrespective of macrovascular status. Critically, a significant positive correlation between IFN- γ and total serum cholesterol was uniquely identified within the CVD-complicated subgroup ($r = 0.336$, $p = 0.034$), absent in the uncomplicated cohort ($p = 0.816$). No other significant associations were detected between IFN- γ and triglycerides, LDL, HDL, or CRP in either subset (all $p > 0.05$).

Table 4. Bivariate correlation analysis of serological and cardiometabolic parameters.

Parameters	SLE with CVD r	SLE with CVD P	SLE without CVD r	SLE without CVD P
A. CRP (mg/L) — Spearman's rank correlation				
Anti-dsDNA (IU/mL)	0.059	0.717 NS	0.249	0.122 NS
B. IFN-γ (pg/mL) — Pearson's correlation				
Cholesterol (mg/dL)	0.336	0.034*	0.038	0.816 NS
Triglycerides (mg/dL)	0.219	0.175 NS	0.173	0.286 NS
LDL (mg/dL)	0.184	0.257 NS	-0.178	0.272 NS
HDL (mg/dL)	0.086	0.598 NS	0.137	0.399 NS
CRP (mg/L)	0.083	0.612 NS	0.202	0.212 NS
Anti-dsDNA (IU/mL)	0.326	0.040*	0.373	0.018*

Spearman's rank correlation (CRP analyses); Pearson's correlation (IFN- γ analyses). * $p < 0.05$; NS = Not Significant ($p \geq 0.05$).

Table 5 presents ROC curve analysis confirming the significant diagnostic utility of IFN- γ in differentiating SLE from healthy controls ($p = 0.001$ for both comparisons). Using Youden's index to determine optimal cut-off thresholds, an IFN- γ cut-off of > 162.29 pg/mL identified the CVD-complicated SLE cohort with high accuracy (AUC = 0.973), yielding 95.0% sensitivity and 97.5% specificity. Similarly, a threshold of > 154.04

pg/mL reliably discriminated the uncomplicated SLE group (AUC = 0.945) with 92.5% sensitivity and 95.0% specificity. High positive and negative predictive values ($> 92\%$ across all subsets) unequivocally validate baseline IFN- γ as a highly sensitive and specific serological biomarker for SLE pathogenesis, with maximal predictive precision in patients harbouring concurrent macrovascular complications.

Table 5. ROC curve analysis of systemic IFN- γ for discriminating SLE clinical phenotypes from healthy controls.

ROC Parameter	SLE with CVD vs. Healthy Control	SLE without CVD vs. Healthy Control
Cutoff value	> 162.29 pg/mL	> 154.04 pg/mL
P-value	0.001	0.001
Sensitivity (%)	95.0%	92.5%
Specificity (%)	97.5%	95.0%
PPV (%)	97.4%	94.9%
NPV (%)	95.1%	92.7%
AUC (95% CI)	0.973 (0.945 – 1.000)	0.945 (0.896 – 0.994)

Optimal cut-off values determined by Youden's index. AUC = Area Under the Curve; PPV = Positive Predictive Value; NPV = Negative Predictive Value; CI = Confidence Interval.

Figure 2: ROC curves for IFN- γ as a discriminator of SLE from healthy controls. Blue curve: SLE with CVD vs. healthy controls (AUC = 0.973). Green curve: SLE without CVD vs. healthy controls (AUC = 0.945). The pronounced leftward and upward shift of both

curves away from the diagonal reference line visually corroborates the exceptional diagnostic accuracy of IFN- γ , with maximum predictive precision in patients with concurrent macrovascular complications.

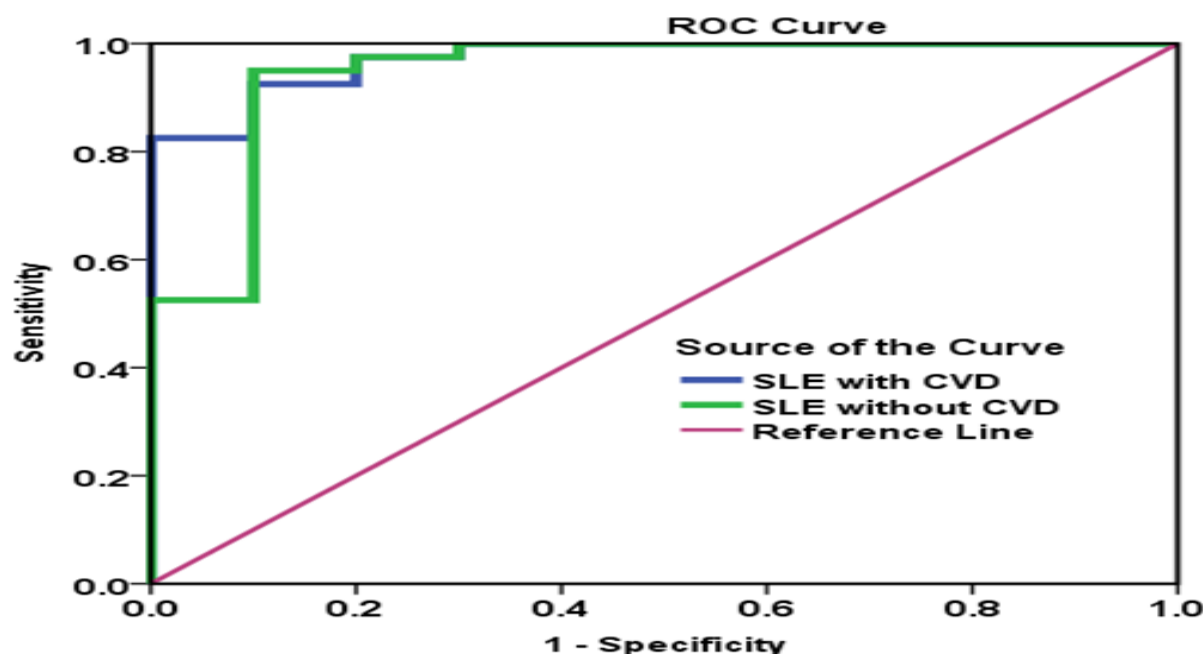


Figure 2: ROC curves for IFN- γ . Blue curve: SLE with CVD vs. healthy controls (AUC = 0.973). Green curve: SLE without CVD vs. healthy controls (AUC = 0.945).

Figure 3: Bar chart illustrating mean serum anti-dsDNA concentrations across the three study groups. Elevated levels in both uncomplicated SLE (3.13 IU/mL) and CVD-complicated SLE (2.84 IU/mL) compared to healthy controls (2.23 IU/mL) confirm the autoimmune specificity of anti-dsDNA as a disease marker however, comparable mean values between the two patient subsets indicate that gross anti-dsDNA levels do not stratify the clinical phenotypes based on macrovascular status.

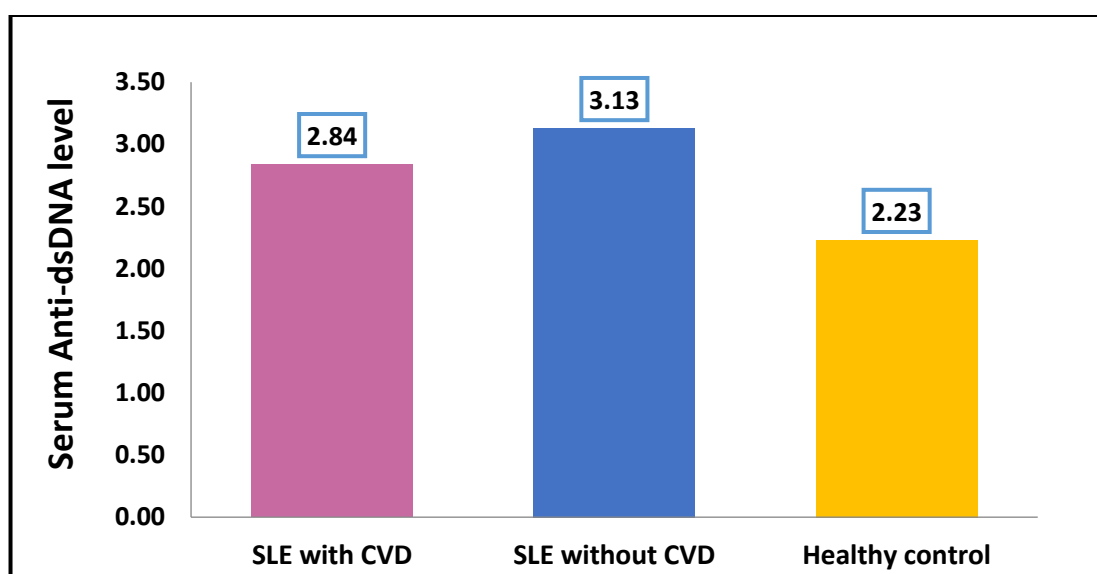


Figure 3: The means level of Anti-dsDNA in patients and control groups.

DISCUSSION

The primary objective of this study was to elucidate the specific serological signatures associated with macrovascular complications in SLE. The salient finding of our investigation is the discriminative capacity of the Th1-signature cytokine, IFN- γ , which successfully stratified SLE patients with concurrent CVD from those with uncomplicated SLE, an area where traditional inflammatory and lipemic markers demonstrated clinical limitations, consistent with emerging contemporary literature(12,13). Our results demonstrated that traditional autoimmune and acute-phase indices—specifically anti-dsDNA autoantibodies and CRP, while significantly elevated compared to healthy baselines, did not differentiate between the clinical SLE phenotypes. Furthermore, the lack of correlation between CRP and anti-dsDNA in our sub-cohorts suggests that the magnitude of the acute-phase inflammatory response operates independently of the specific autoantibody load. This dynamic implies that the generalized acute-phase response and global autoantibody burden may merely reflect the baseline chronicity and severity of the underlying autoimmune dysregulation, rather than acting as specific triggers for the atherogenic tipping point(14). Macroscopic lipid profiles (total cholesterol, triglycerides, LDL, HDL) were statistically uniform across all evaluated cohorts. Consequently, traditional gross lipemic indices failed to serve as differentiating serological markers for concomitant macrovascular complications in this study. It is hypothesized that qualitative micro-alterations in lipoproteins, such as oxidation and modified efflux capacities, rather than quantitative gross serum levels, perhaps play a more definitive and aggressive role in SLE-driven atherogenesis (15,16).

In contrast to these traditional markers, the systemic upregulation of IFN- γ uniquely differentiated the CVD-complicated SLE cohort. This demonstrates a progressive,

stepwise escalation of Th1-mediated inflammatory burden that strongly distinguishes basal autoimmunity from subsequent cardiovascular pathogenesis. IFN- γ is a principal mediator of the Th1 inflammatory axis and may critically drive localized endothelial dysfunction, accelerate macrophage polarization into foam cells, and destabilize atherosclerotic plaques(17,18).

Crucially, our bivariate analysis unveiled a significant positive correlation between IFN- γ and total cholesterol exclusively within the CVD-complicated subgroup. This suggests that the pro-atherogenic intersection of this specific cytokine may be primarily mediated through dysregulated total cholesterol metabolism rather than broader lipidemic shifts. This intricate cardiometabolic interplay strongly implies that localized Th1-cytokine expression perhaps acts synergistically with circulating sterols to exacerbate pro-atherogenic inflammatory loops, a mechanism that is increasingly supported by novel immune-metabolic paradigms (19,20).

The diagnostic utility of IFN- γ was validated by our ROC curve analysis, which demonstrated strong discriminative accuracy. Such statistical precision underscores the potential of this specific cytokine as a reliable predictive screening tool in rheumatological clinics. Notably, our subgroup analyses indicated that this pronounced cytokine expression remained independent of both familial predisposition and disease duration. This finding implies that macrovascular complications in SLE may be driven by distinct immune-metabolic pathways rather than the mere temporal accumulation of autoimmune damage over the years (21).

Limitations of the Study

Several methodological limitations warrant acknowledgment. First, the cross-sectional, single-center design precludes causal inferences and limits geographic generalizability. Second, while ROC analysis

yielded unusually high AUC values for IFN- γ , the modest specimen size may have caused statistical overfitting, necessitating validation in larger, multi-center longitudinal cohorts. Third, our analysis was restricted to systemic cytokines, lacking localized vascular tissue assessment. Furthermore, the absence of multivariate regression prevents isolating the independent effect of BMI variance on systemic inflammation. Finally, logistical constraints precluded incorporating detailed pharmacological histories and concurrent SLEDAI scores. Although this omission could influence baseline cytokine profiles, applying strict exclusion criteria for recent therapeutic alterations partially mitigated this variance.

Recommendations: Future prospective, multi-center longitudinal studies with larger sample sizes are highly recommended to validate the established IFN- γ diagnostic thresholds and investigate the sequential molecular mechanisms linking Th1-mediated inflammation to accelerated atherogenesis in SLE patients.

CONCLUSION

In conclusion, this study demonstrates that systemic quantification of the Th1-signature cytokine, IFN- γ , serves as a strong discriminative serological biomarker for identifying concurrent cardiovascular complications in patients with SLE. While traditional acute-phase reactants (CRP), specific autoantibodies (anti-dsDNA), and conventional lipid panels reflect baseline autoimmune activity, they did not adequately stratify macrovascular risk within this clinical cohort. The significant upregulation of systemic IFN- γ , alongside its specific correlation with total serum cholesterol in the CVD-complicated group, suggests a potential interplay between Th1-mediated inflammatory pathways and circulating sterols in accelerating atherogenesis. Furthermore, this distinct immune-metabolic

profile appears to operate independently of disease duration and familial predisposition.

Integrating baseline IFN- γ profiling into rheumatological assessments may offer valuable additional diagnostic insights over standard indices, potentially aiding in the earlier detection of vulnerable SLE phenotypes. Given the cross-sectional nature of this study, future longitudinal, multi-center research is warranted to validate these diagnostic thresholds and elucidate the sequential mechanisms linking cytokine-driven autoimmunity to cardiovascular events.

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Ethical approval

The present study which is conducted by authors (Zahraa Saeed Shandil and Eman Hasani Shbait AL-Salami) was approved by the local Institutional Ethics Committee of the Faculty of Medicine at University of Kufa (Approval Protocol No. 69693). Written informed consent was systematically obtained from all patients and healthy volunteers prior to their enrollment and any specimen collection.

Statement of Permission and Conflict of Interests

The authors declare that there is no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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