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Research Article

Comparative Study of Serum and Urine Cell Free DNA, miRNA-27b, as Novel Biomarkers with INF-Y for Assessing Systemic Lupus Erythematosus and Lupus Nephritis Activity

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Key Words

microRNA, miRNA-27b, Cell-free DNA (cf-DNA), interferon gamma (INF-γ), Systemic lupus erythematosus (SLE). Lupus Nephritis (LN)

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Abstract: Systemic lupus erythematosus (SLE) disease a condition as one of the causes of loss of tolerance to several autoantigens triggering the involvement of the immune system to the destruction of tissues. One of the most disabling symptoms of systemic lupus erythematosus (SLE) refers to lupus nephritis (LN). It does also have a frequency-of-occurrence that is dependent on some level of kidney inflammation. The immune complexes accumulated in the mesangial, subepithelial and subendothelial parts of the glomeruli cause the kidneys damage by causing several inflammatory, proliferative and fibrous pathways in these regions. The patterns of deposition of different immune complexes are associated with different lupus types of nephritis (LN) and their clinical chemistry symptoms. This research paper set out to assess the use of blood and urine cell-free DNA (cfDNA) as a non-invasive lupus nephritis biomarker in patients. In addition, serum microRNA-27a and serum INF-Y evaluated whether there were any correlations with the severity of the disease. The participants in this study were divided into three groups and the number of participants was 106. The initial group was patients who had SLE and had 6 males and 27 females (n=36). Moreover, 12 men, 24 women were patients with nephritis (n=35). Lastly, 11 men, 24 women were those who were not affected by SLE or nephritis (n=35). Three groups participated in a

cross-sectional study was conducted based on 106 participants was classified as follows: 36 patients with SLE (6 men and 27 women), 35 patients with lupus nephritis (12 men and 24 women), and 35 as a control group (11 men and 24 women) without a history of systemic disease. The study was conducted from January 2024 to September 2024. All participants had been selected from Al-Sader Teaching Medical City in Najaf province and private labs. The study involved three groups: lupus nephritis (n=35), systemic lupus erythematosus (SLE, n=36), and healthy controls (n=35). The lupus nephritis group included 12 males and 23 females, while the SLE group had 9 males and 27 females. The healthy control group had 11 males and 24 females. Across all groups (n=106), there were 32 males and 74 females, showing a case-control ratio of 2.03:1 for 71 diseased individuals compared to 35 healthy controls. sex distribution did not differ significantly among the groups (p=0.685). The age distribution in the study groups showed the following: lupus nephritis (n=35) had 9 under 20, 11 aged 20-30, 9 aged 31-40, 5 aged 41-50, and 1 over 50. The systemic lupus erythematosus (SLE) group (n=36) included 4, 9, 14, 8, and 1 in the respective age ranges. The healthy control group (n=35) comprised 1, 6, 15, 13, and 0 individuals. In total (n=106), there were 14 participants under 20, 26 aged 20-30, 38 aged 31-40, 26 aged 41-50, and 2 over 50. Statistical analysis indicated significant association between age group and group membership ($p = 0.271$). The levels of pro-inflammatory cytokine INF-T was evaluated across the three studied groups, where the median levels were significantly higher in both the lupus nephritis group (70.00, IQR = 429.00) and the SLE group (48.00, IQR = 386.75) compared to the healthy control group (0.70, IQR = 0.40), with an overall statistically significant difference between groups ($p < 0.0001$). The fold change of miRNA-27b was markedly reduced in the lupus nephritis group (median = 0.00221, IQR = 0.00420) compared to both the SLE group and healthy controls, which showed nearly identical low levels (median = 0.00017, IQR = 0.00080 for both), also yielding a highly significant p-value ($p < 0.0001$). Serum cell-free DNA (cfDNA) levels between patients with lupus nephritis and SLE, expressed as fold change relative to healthy controls. In this analysis, the healthy control group showed no detectable Ct values in RT-PCR, indicating minimal or undetectable cfDNA levels; therefore, a default fold change value of 1 was assigned to the control group for quantification purposes. The lupus nephritis group had a median cfDNA fold change of 0.353 (IQR = 0.408), while the SLE group showed a median of 0.291 (IQR = 0.000). However, the difference between the two patient groups was not statistically significant ($p = 0.573$). The healthy control group again showed no detectable Ct values in qPCR, indicating undetectable cfDNA levels. The lupus nephritis group had a median urine cfDNA fold change of 0.0306 (IQR = 0.073), while the SLE group showed a higher median of 0.1518 (IQR = 0.162). A statistically significant difference was observed between the two groups ($p = 0.036$). In Conclusion This research illustrates the potential of cell-free DNA (cfDNA) and specific microRNAs (miRNA-27b) as non-invasive biomarkers for assessing lupus nephritis (LN) in patients with systemic lupus erythematosus (SLE). The study presents evidence of increased levels of pro-inflammatory cytokine(INF-Y) in both SLE and LN groups when compared to healthy controls, highlighting a strong inflammatory response in these conditions. Furthermore, the expression levels of miRNA-27b was significantly reduced in LN and SLE patients, suggesting these microRNA might serve as indicators of disease activity and severity. The investigation also assessed cfDNA levels in blood and urine samples. While serum cfDNA levels showed no significant differences between LN and SLE groups, urine cfDNA levels were notably higher in the SLE cohort compared to LN patients, indicating that urine cfDNA could be a more sensitive biomarker for distinguishing between these two groups. These findings underscore the importance of incorporating cfDNA and specific microRNA markers into the diagnostic and prognostic approaches for lupus nephritis, highlighting their relevance in non-invasive disease monitoring. Future research should focus on larger cohort studies and the molecular roles of these biomarkers in the pathogenesis of lupus nephritis and systemic lupus erythematosus. This approach could lead to more accurate, personalized treatment strategies and improved patient outcomes.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multifaceted autoimmune connective tissue disorder characterized by the generation of diverse autoantibodies targeting DNA and other cellular components. It is a typical disease with diverse clinical symptoms that may various organs and systems[1].

It exhibits a diverse array of clinical manifestations. Certain individuals solely exhibit arthralgias and rashes, while others endure significant multi-organ involvement, including vasculitis or nephritis[2]

Lupus nephritis impacts a significant percentage of SLE patients, with estimates indicating that renal involvement occurs in between 25% to 60% of instances. This syndrome is marked by various morphological features and clinical presentations, potentially resulting in severe consequences, including end-stage renal disease (ESRD) in a significant proportion of individuals[3].

The pathogenesis of lupus nephritis is intricate, chiefly characterized by the accumulation of immunological complexes in the kidneys. Immune complexes are constituted by autoantibodies that may recognize diverse antigens, including DNA. This immunological reaction may cause considerable inflammation and tissue damage, perhaps culminating in renal failure if not properly treated[4].

Renal biopsy is the conclusive diagnostic method for lupus nephritis, providing comprehensive insights into histological classifications and disease severity. Nonetheless, its invasive characteristics and related hazards, including hemorrhage, restrict its regular application for assessing disease progression or therapy efficacy. Serial biopsies are unfeasible for long-term care, requiring the creation of dependable non-invasive diagnostic instruments[5].

Traditional indicators such as proteinuria and serum creatinine lack specificity and do not accurately represent histopathological alterations, highlighting the necessity for innovative alternatives[6].

Unlike other biological sample sources, such as serum or tissue, urine collection is non-invasive, facilitating regular monitoring, and may be self-collected, transported, and stored with ease. Moreover, urine biomarkers appear to be more advantageous than serum markers in the investigation of LN, as they originate from the urinary system's tissues, thereby accurately reflecting its current clinical condition. Consequently, urine serves as a promising medium for identifying possible biomarkers in the investigation of lupus nephritis (LN)[7].

Research on cfDNA in SLE has progressed from initial research concentrating on the detection and quantification of cfDNA to subsequent investigations linking cfDNA levels with disease activity, progression, and therapy Concerning MicroRNA-27b (miR-27b) recent research suggest that miR-27b may have a significant role in autoimmune illnesses, especially systemic lupus erythematosus (SLE) and its renal manifestation, lupus nephritis (LN). Research indicates that miR-27b plays a role in regulating inflammatory responses and influences the survival and function of immune cells, which are essential in the pathogenesis of systemic lupus erythematosus (SLE)[8].

Moreover, miR-27b has been recognized as a regulatory site in lipid metabolism, potentially affecting the inflammatory mechanisms linked to lupus nephritis[9]. Moreover, research has emphasized the potential of miR-27b as a biomarker for disease activity in SLE patients, especially those with renal involvement, indicating its usefulness in detecting disease progression and therapy responses[10]

In recent years, the prevalence of lupus nephritis has markedly risen in Iraq, potentially attributable to many factors. Furthermore, managing this ailment has grown challenging in aggravated instances. This study was intended to determine the amount of illness dissemination and to evaluate various parameters as potential prognostic biomarkers and therapeutic targets. The aim of the present research is to assess the usefulness of cell-free DNA (cfDNA) in urine and blood samples as a non-invasive biomarker for patients with lupus nephritis, along with the detection of serum fold change of miRNA-27b, and serum levels of INF-γ, to find out the relation with disease severity.

MATERIALS AND METHODS

Patient: A cross-sectional study was conducted based on 106 participants was classified as follows: 36 patients with SLE (6 men and 27 women), 35 patients with lupus nephritis (12 men and 24 women), and 35 as a control group (11 men and 24 women) without a history of systemic disease. The study was conducted from January 2024 to September 2024. All participants had been selected from Al-Sader Teaching Medical City in Najaf province and private labs. The age range of the enrolled patients was 13 to 67 years, which includes 65 females and 8 males. The nephrologist rendered a clinical diagnosis for

patients suspected of having lupus. Patients with lupus were personally interviewed using an anonymous questionnaire that included inquiries about age, sex, family history, medication history, sickness severity, recurrence, length of diseases, and other autoimmune conditions. Systemic Lupus Erythematosus Disease Activity Index 2000 (SLE-DAI-2 K) was applied to outline the activity. ELISA was employed to evaluate INF-y. Real-time PCR was employed to quantify the expression levels of miRNA-27b in whole blood and cell-free DNA in serum and urine. Where 5 ml of blood sample from each participant has been collected for detection of INF-y, miRNA-27b and cell free DNA, also 5 ml of urine samples were collected for detection of cell free DNA and other urine parameters.

The ethical approval committee of the College of Medicine, University of Al-Qadisiyah College of Medicine, sanctioned this study. Informed consent was acquired from every patient participating in the current investigation.

Blood Sampling Protocol: Each participants had a vein puncture by using disposable syringes and aseptic conditions to obtain 5 milliliters of blood. Each sample was given 3 milliliters in a sterile gel tube, which was then left to clot for a few minutes to an hour at room temperature. After 10-minute centrifugation at 2500 rpm, the separated serum was stored in Eppendorf tubes and immediately frozen at -20°C until it was needed for ELISA testing to determine the levels of INF-y, as well as, cell free DNA molecule detection by qPCR. The remaining (2ml) of blood was transported into a Eppendorf tube with triazole and kept at -70°C until analyzed with real-time PCR for measuring the expression levels of miRNA-27b in whole blood.

Urine Sample Protocol: 10 ml of urine were collected by using disposable cup of urine to physical, chemical, and direct examination after 10-minute centrifugation at 5000 rpm). 2ml of urine was separated and stored in Eppendorf tubes and frozen at -20°C to cell free DNA molecule detection by qPCR. Also, 0.5 ml of urine samples was kept in Eppendorf tube with triazole (0.5 ml) at - 70°C until analyzed with real-time PCR for measuring the expression levels of cell-free DNA .

Determination of Cell Free DNA and Beta-globin Reference Gene Expression in Samples by RT-Qpcr cf-DNA Extraction: The samples of serum and urine were prepared from fresh or frozen human samples by centrifugation for 10 min at 2000 × g. serum was removed from the cell pellet, taking care to avoid leukocyte carryover. Some samples were frozen and stored for several days to weeks at -80°C.

DNA Isolation: Samples were re-centrifuged for 3 min at 11,000 × g to remove any particulate matter, and the cleared supernatant was further processed. The isolation of DNA from cell free samples was performed essentially according to the manufacturer's instructions. The kit utilizes the benefits of a new column type which directs the sample to a very small silica membrane. This allows for elution of DNA in 5-30 µL.

200 µL (rapid protocol) or 240 µL (high-sensitivity protocol) serum sample was mixed with 1.5 volume binding buffer. The mixture was applied to the spin column and spun for 30 sec at 11,000 × g. After two subsequent washing steps (each with 500 µL wash buffer; 30 sec. 11,000 × g for the first wash and 3 min 11,000 × g for the second wash), the DNA was eluted with 20 µL (rapid protocol) and for some experiments additionally heat-treated for 8min at 90°C (high-sensitivity protocol). Proteinase K treatment was not utilized within the NucleoSpin serum XS experiments described here. The isolated DNA was quantified either with Pico Green_ luorescent dye, or a quantitative real-time PCR with TaqMan_ probe for a 135-bp single-copy gene (human endogenous retrovirus). samples were quantified using a real-time PCR with SYBR_ Green and an 81-bp single-copy beta globin target. There used the primers

5- CAACCTCAAACAGACACCAT -3 and 5_CACGTTCACCTTGCCCCACA- 3[11,12]

PCR Technique: GoTaq® Green Master Mix (Promega-USA) was used to prepare PCR according to the type and target region of DNA Figure 1.

Measuring the Expression Levels of Mature miRNA-27b by Real-time PCR (Q-PCR): Used the Applied Biosystems Step One Plus to do quantitative real-time polymerase chain reaction (Q-PCR), which looks at the fold change of miRNA-27b in the whole blood of patients with SLE, LUPUS NEPHRITIS, and healthy controls. Also employed real-time PCR to determine the relative expression levels of the housekeeping gene (U6) (reference gene) in both patients and healthy controls using the one-step approach.

Extraction of Micro RNA from Whole Blood: By using the kit of Trans Zol Up, micro RNA was extracted from whole

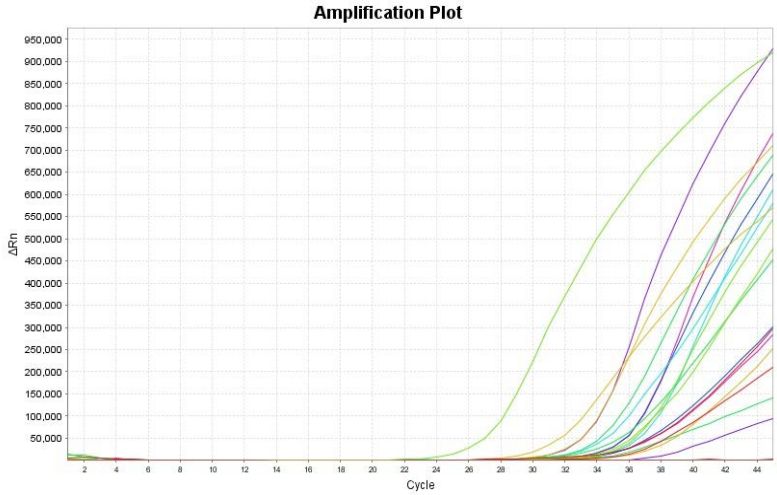

Fig. 1: cfDNA Amplification Plot

blood samples by the use of the company's instructions, which were as follows: The blood samples had been thawed that were stored in Eppendorf tube with triazole in 70c. Then, 100 µl of chloroform was added to each tube, and the tubes were shaken firmly by hand for 30 seconds. Incubated at room temperature for 3 minutes. The sample was centrifuged at 10000 rpm, 4°C, for 15 minutes. The supernatant was transferred into a fresh Eppendorf tube, and 250 µl of isopropanol was added. Following four or five tube inversions, the mixture was combined and allowed to sit at room temperature for ten minutes. Then, centrifuged for 10 minutes at 10,000 rpm and 4°C. The supernatant was discarded, 500 µL of 75% ethanol was added, and it was vortexed. All sample was centrifuged for five minutes at 4°C and 7500 rpm. The RNA pellet was allowed to air dry while the supernatant was disposed to dissolve the RNA pellet, 30µl DEPC water was added to each sample, and the extracted RNA sample was stored at -70.

Calculating the Purity and Concentration of Total RNA: A Nanodrop spectrophotometer (BIODROP. BRITISH) was used to evaluate the isolated genomic RNA. It measured the absorbance at 260/280 nm to determine the RNA purity and concentration.

The reference gene or housekeeping gene or endogenous control gene selected by finding the best and the more stable reference gene expressed in the serum samples. The best reference gene depends on three parameters: high expression level; stable and expressed among all samples; and then showing of converge expression level among all samplesl[13].

Determination of miRNA-27B and U6 reference gene Expression in Samples by one step RT-Qpcr: GoTaq 1-Step RT-qPCR System combines GoScrip Reverse Transcriptase and GoTaq qPCR Master Mix in a single-step real-time amplification reaction. The system, optimized for RT-qPCR, contains a proprietary fluorescent DNA binding dye, Sybr Green Dye. The system enables the detection of RNA expression levels using a one-step RT-qPCR method:
GoTaq® 1-Step RT-qPCR component, total RNA, primers and Nuclease-free water were all thawed on ice and each solution was mixed well.

Gene Expression Calculation (Gene Fold): Two methods are available for analyzing qPCR data: absolute and relative Quantification. Absolute quantification uses Schmittgen and Livak's standard curve to identify the amount of input gene. Pfaffl, on the other hand, uses relative quantification to as certain changes in gene expression about a reference gene sample[14].

RQ = 2-(ΔΔCT)

The gene fold was calculated firstly by collecting CT (CT - cycle threshold) average value from real time PCR device for each sample then ΔCT value was calculated as follows:

ΔCT = CT (gene of interest) – CT (reference gene)

ΔCT is the difference in CT values for the gene of interest and reference gene for a given sample. This is essential to normalize the gene of interest to a gene, which is not affected by experiment.
Calculating ΔΔCT value is found as follows:

ΔΔ CT = Δ CT (patient sample) – Δ CT (healthy control)

After calculating ΔΔ CT for all samples, the final equation is taken to calculate the gene expression (fold change) as follows:

Fold gene expression RQ = $_2$-(ΔΔCT)

Statistical Analysis: The SPSS statistical package for the Social Sciences was used to analyze the results (version 20.0 for windows, SPSS, Chicago, IL, (USA).

RESULTS AND DISCUSSIONS

The present study revealed that SLE lupus nephritis is more prevalent in female than in male. Nonetheless, there was no significant difference in the frequency distribution of patients and control participants based on gender (P = 0.685).

Contemporary literature consistently reports a female predominance in systemic lupus erythematosus (SLE). where, the present results are consistent with studies reported in a 2025 scoping analysis of 81 research indicated female-to-male ratios ranging from 4:1 to 11:1, highlighting that men had later illness onset and more severe organ damage[15]. A retrospective study done by[16] referred to an infection risk in systemic lupus erythematosus (SLE) reaffirmed that the condition predominantly impacts women, with a female-to-male ratio of approximately 9:1, and these results agree with the study results .Another cohort research involving 1,048 biopsy-confirmed lupus nephritis patients from 1996 to 2018 indicated that 17% were male and 83% were female; male patients exhibited elevated blood pressure, more severe histopathological findings, and considerably higher mortality rates (24.2% compared to 13.4% in females)[17]. The results indicate that, globally, males typically represent approximately 10-20% of SLE cases, with female preponderance most pronounced during reproductive years and considerably diminished in children and elderly adults.

In addition to epidemiology, novel ecological reviews have investigated the reasons for the disproportionate impact of SLE on women. A 2024 study in Biology of Sex Differences indicated that women may be up to 14 times more vulnerable to systemic lupus erythematosus (SLE), attributing this disparity to X-chromosome dosage. It noted that supernumerary X-chromosome syndromes (XXY, XXX) elevate SLE risk, whilst Turner syndrome (XO) mitigates it[18]. Evading X-chromosome inactivation permits the over expression of genes like TLR7 and TLR8 in female immune cells, hence augmenting interferon-a production and autoimmune reactions, in contrast, androgen signaling and the absence of a second X chromosome may confer protection to males. The sex-specific immunogenetic variables, in conjunction with hormonal impacts such as estrogen's role in enhancing antibody production, elucidate the pronounced female bias[16] Table 3.

The current study presents the age distribution among three groups-lupus nephritis, systemic lupus erythematosus (SLE), and healthy controls-and identified no statistically significant correlation between age group and illness status (p = 0.271). This corresponds with recent studies showing that although SLE and lupus nephritis can manifest at any age, most instances generally aggregate between the second and fourth decades of life. Also[17] found that lupus nephritis was prevalent in younger patients, with 63.5% of those affected being under 35 years old. The overall findings align with the observation that younger individuals are more likely to develop lupus nephritis. This result also agrees with studies found that Systemic lupus erythematosus predominantly occurs in adults aged 20 to 40, with research indicating that 64% of patients are within this demographic[18,19].

Notably, the healthy control group exhibited a concentration within the 31-50 year age range (71.4%), which is not atypical and may indicate general population demographics rather than disease-specific patterns[20] Table 4.

The findings indicate significantly increased INF-γ levels in both lupus nephritis (LN) and systemic lupus erythematosus (SLE) groups when contrasted with healthy controls, with LN patients exhibiting the highest median levels. INF-γ is an important Th1 cytokine that activates macrophages, helps deliver antigens, and boosts inflammatory signaling in autoimmune disorders. The increased levels of INF-γ in LN patients correspond with previous research indicating its involvement in promoting renal inflammation through the activation of macrophages and mesangial cells, resulting in glomerular damage and fibrosis[21].

The difference between LN and SLE absent nephritis indicates a heightened Th1 immune activation in renal-involved disease, aligning with the findings of[22] which demonstrated elevated INF-γ expression in kidney biopsy tissues of LN patients. Nevertheless, certain investigations with milder SLE cases devoid of renal involvement did not see a pronounced rise of INF-γ, potentially attributable to a reduced systemic inflammatory burden or a shorter disease duration within those cohorts[23] Table 5.

miRNA-27b was markedly reduced in the lupus nephritis group (median = 0.00221, IQR = 0.00420) compared to both the SLE group and healthy controls, which showed nearly identical low levels (median = 0.00017, IQR = 0.00080 for both), also yielding a highly significant p-value ($p < 0.0001$).

Bai[24] and Xu[25] pointed that data on human LN regarding miR-27b are more scarce compared to other miRNAs; yet, mechanistic and translational

Table 1: GoTaq® 1-Step RT-qPCR reaction was prepared

Component	Volume Final	Concentration
GoTaq® qPCR Master Mix, 2X	10 µl	1X
GoScript™ RT Mix for 1-Step RT-qPCR (50X)	0.4 µl	1X
Forward Primer (20X)	0.6 µl	300 nM
Reverse Primer (20X)	0.6 µl	300 nM
MgCl2	1.6 µl	25 mM
RNA template	5 µl	100 ng
Nuclease-Free Water	1.8 µl	-
Total	20 µl	-

Table 2: RT-qPCR reactions were performed using the cycling program

Step	Temperature	Duration	Cycles
Reverse transcription	37 C°	15 min	1
RT inactivation/Hot-start activation	95 C°	10 min	1
Denaturation	95 C°	10 sec	50
Annealing	58 C°	30 sec	
Extension and data collection	72 C°	30 sec	

Table 3: Gender Distribution of Three Studied Groups

Group	Gender		Total	P- Value
	Male	Female		
Lupus Nephritis	12(34.3 %)	23 (65.7 %)	35 (100%)	685 (NS)
SLE	9 (25 %)	27 (75 %)	36 (100%)	
Control	11 (31.4 %)	24 (68.6 %)	35 (100%)	
Total	32 (30.2 %)	74(68.8%)	106(100%)	

Table 4: Age Distribution of Three Studied Groups

Group	Age Group					Total	P-value
	<20	20–30	31–40	41–50	>50		
Lupus Nephritis	9	11	9	5	1	35	0.271 (S)
	(25.7%)	(31.4%)	(25.7%)	(14.3%)	(2.9%)	(100%)	
SLE	4	9	14	8	1	36	
	(11.1%)	(25.0%)	(38.9%)	(22.2%)	(2.8%)	(100%)	
Healthy Control	1	6	15	13	0	35	
	(2.9%)	(17.1%)	(42.9%)	(37.1%)	(0.0%)	(100%)	
Total	14	26	38	26	2	106	
	(13.2%)	(24.5%)	(35.8%)	(24.5%)	(1.9%)	(100%)	

Table 5: Evaluation of INF- γ

Cytokine	Group	N	Median	IQR	P- value
INF- γ	lupus nephritis	35	70.00	429.00	0.0001 (S)
	SLE	36	48.00	386’75	
	healthy control	35	0.700	0.40	

Table 6: Fold Change Expression of miRNA-27b in Studied Groups

Fold Change	Group	N	Median	IQR P- value	
miRNA-27b	LN	35	0.00220703	0.004196	0.0001 (S)
	SLE	36	0.00016697	0.000800	
	H.C	35	0.00016697	0.000800	

Kruskal-Wallis Test, with 0.05 Significance Level, S= Significant

Table 7: Diagnostic Accuracy Characteristics of miRNA-27b

Disease	Characteristic	Healthy Control (n)	Patients (n)
Lupus nephritis	Cut off	0.0490	
	> 0.0490	0	35
	> 0.0490	19	0
	AUC (95% CI)	99.7% (0.989-1.00)	
	Sensitivity	100 %	
	Specificity	100 %	
SLE	Cut off	0.003831	
	> 0.003831	0	36
	> 0.003831	19	0
	AUC (95% CI)	100% (1.00-1.00)	
	Sensitivity	97.1 %	
	Specificity	100 %	

studies suggest a protective and anti-fibrotic function. miR-27b-3p mitigates renal fibrosis by regulating STAT1, but in lupus nephritis models, a circRNA (circELK4) sequesters miR-27b-3p, thereby enhancing STING/IRF3/type-I-IFN signaling, which exacerbates inflammation and damage. The statistics indicate that diminished functional miR-27b activity would promote renal inflammation and fibrosis, consistent with these results overall "sub-unitary" fold-change values (<1) across groups Table 6.

The present study observed that it is analysis demonstrates nearly flawless discrimination: in LN, a

Table 8: Comparison of Serum cf DNA in Lupus nephritis and SLE Patient Groups

Group	N	Median	IQR	P-value
Lupus Nephritis	35	0.353	0.408	0.573 (S)
SLE	36	0.291	0.000	

Mann-Whitney U Test, with 0.05 Significance Level, NS= non-Significant

Table 9: Comparison of Urine cf DNA in Lupus Nephritis and SLE Patient Groups

Group	N	Median	IQR	P-value
Lupus Nephritis	35	0.0306	0.073	0.036 (S)
SLE	36	0.1518	0.162	

Mann-Whitney U Test, with 0.05 Significance Level, S= Significant

Table 10: Diagnostic Accuracy Characteristics of cf DNA to Discriminate Between SLE and Lupus Nephritis Patients

Characteristic	SLE (n)	Lupus nephritis (n)
Cut off	0.115	
> 0.115	21	30
> 0.115	15	5
AUC (95% CI)	64.5% (0.517-0.774)	
Sensitivity	88.6 %	
Specificity	41.7 %	

Table 11: Diagnostic Accuracy Characteristics of Serum cf DNA to Discriminate Between SLE and Lupus Nephritis Patients

Characteristic	SLE (n)	Lupus nephritis (n)
Cut off	0.135607	
< 0.135607	32	26
> 0.135607	4	9
AUC (95% CI)	46.1% (0.517-0.774)	
Sensitivity	74.3 %	
Specificity	27.5 %	

Table 12: Correlation Among Disease Severity of Two Disease Groups (Lupus Nephritis And SLE) and Molecular Markers

Variable	Statistics	Severity (lupus nephritis)	Severity (SLE)
Severity	Correlation Coefficient	1	1
	P- value	.	.
	N	35	36
fold chin of cf DNA in serum	Correlation Coefficient	-.021-	-.258-
	P- value	0.906 (NS)	0.128 (NS)
	N	35	36
fold chin of cf DNA in urine	Correlation Coefficient	0.126	-.342-
	P- value	0.471(NS)	0.041(S)
	N	35	36
miRNA-27B fold change	Correlation Coefficient	-.172-	-.070-
	P- value	0.325 (NS)	0.685 (NS)
	N	35	36

threshold of around 0.0490 categorized all 35 LN cases as positive and all 35 controls as negative (AUC = 0.997; 95% CI 0.989-1.00). In systemic lupus erythematosus (SLE), a reduced cut-off (~0.003831) attained 97.1% sensitivity and 100% specificity, with an area under the curve (AUC) of around 1.00; therefore, Xu[25] indicated a highly significant distinction in the detected miRNA-27b signal between patients and controls in your group. Biologically, this separation is feasible if disease mechanisms inhibit miR-27b in pertinent renal and immune cell compartments; at your thresholds, "test-positive" likely indicates diminished miR-27b expression compared to controls. The present result came agree with Bai[26] that pointed to a mechanistic basis exists for miR-27b-3p's protective role in LN. CircELK4 in kidney cells sequesters miR-27b-3p, facilitating the activation of the STING/IRF3 type-I interferon pathway. Experimental upregulation of miR-27b-3p diminishes inflammatory signaling and apoptosis in lupus nephritis models. In addition to interferon signaling, miR-27b-3p has anti-fibrotic properties in kidney injury through the inhibition of STAT1. Collectively, these data indicate that diminished levels of circulating or urine miR-27b correlate with the activity of lupus nephritis (LN) and systemic lupus erythematosus (SLE).

The current study is consistent with what was indicated by Li[27], although numerous SLE/LN studies emphasize miR-21, miR-29c, miR-150, and miR-146a, members of the miR-27 family, particularly miR-27a, are also included in diagnostic assessments. Furthermore, miR-27b is consistently associated with mechanisms underlying renal fibrosis and immune signaling, notably in diabetic kidney disease and various fibrotic conditions. Biological evidence endorses the utilization of miR-27b as an indicator of kidney inflammation and fibrosis, despite the limited publication of its direct ROC performance in fewer LN populations to date.

Alduraibi and Tsokos, (2024) reported -from a clinical point of view- that miR-27b may serve as a non-invasive tool for differentiating lymphadenopathy or systemic lupus erythematosus from healthy conditions and, crucially, might monitor renal inflammation and fibrosis biology that current serological markers (anti-dsDNA, complement) inadequately reflect. Current evaluations highlight the necessity for biomarkers that reflect intrarenal inflammation and forecast exacerbations or therapeutic responses-functions for which miRNAs and urinary markers are being rigorous examination. An effective miR-27b assay may enhance known indicators and novel urinary proteins (e.g., MCP-1, NGAL, KIM-1, sCD163), where aim of this current study came to align with what was brought by Alduraibi and Tsokos (Table 7).

In this group, serum cfDNA-quantified as fold change relative to healthy controls-showed no significant difference between lupus nephritis (LN) and non-renal SLE (medians 0.353 vs. 0.291; p=0.573). This indicates that the amount of bulk serum cfDNA were measured shows the overall disease level in a similar way for both types.

Numerous studies indicate that circulating cfDNA levels rise in systemic lupus erythematosus (SLE) relative to healthy controls and frequently correlate with overall disease activity. Thereby, the present study agrees with Ibrahim and colleagues, who revealed that elevated cfDNA levels correlate with SLE activity ratings, hence reinforcing the notion that cfDNA serves as a biomarker. Reviews of SLE biomarkers also identify cfDNA as a recurring, although method-sensitive, possibility[28]. Furthermore,[29] discerned certain cfDNA profiles linked to renal involvement, revealing that patients exhibiting atypical cfDNA fragmentation patterns presented with reduced eGFR and more severe lupus nephritis.

The present finding came agreement with[30] who established a notable increase in cfDNA among SLE patients, positively correlating with disease activity indicators such as SLEDAI-2k scores and anti-dsDNA levels. The treatment seemed to diminish cfDNA levels, indicating its usefulness as a biomarker for therapeutic monitoring. These combined data support cfDNA as a promising biomarker for the activity of systemic lupus erythematosus and organ involvement.

The lack of a significant LN-SLE difference aligns with research indicating that cfDNA concentration serves essentially as a rudimentary systemic marker, whereas qualitative characteristics (fragmentomics/ epigenetics) or complexed forms (NET-DNA) provide more phenotype-specific insights[31].

Whereas Truszewska[32] pointed to the certain groups indicate elevated cfDNA levels in severe or renal disease; variations possibly emerge from disease activity, treatment exposure (immunosuppression reduces NETosis/cfDNA), renal function (clearance), and methodological discrepancies among laboratories. The heterogeneity is thoroughly described in recent overviews of LN biomarkers (Table 8).

In this study, urine cfDNA was undetectable by qPCR in healthy controls, but both patient groups had a significant signal. The SLE group had a greater median urine cfDNA fold-change compared to the lupus nephritis (LN) group (0.1518 vs 0.0306; p = 0.036), with a broader dispersion in SLE (IQR 0.162 vs 0.073). This pattern indicates the existence of disease-associated cfDNA in urine and implies group-level variations in the biology or management of extracellular DNA. Urine is progressively utilized as a "liquid biopsy" in lupus nephritis, as it can indicate intrarenal inflammation without the hazards associated with repeated biopsies. Detection may differ according on the target and test sensitivity; however, "undetectable" in controls agree with previous studies indicating little background levels in healthy individuals[33,34].

A significant portion of urine cfDNA is trans-renal, having been filtered from the bloodstream; consequently, variables that reduce free circulating cfDNA, such as immune complex formation, also diminish urinary cfDNA levels. Analytical decisions (nuclear versus mitochondrial targets, fragment length windows, normalization to creatinine, and storage and extraction protocols) can significantly alter absolute values and group rankings-pre-analytical variability is a well-documented factor contributing to inconsistent cfDNA results across studies.

At the selected cutoff of 0.115, cfDNA demonstrates excellent sensitivity but low specificity in differentiating systemic lupus erythematosus (SLE) from lupus nephritis (LN), with an area under the curve (AUC) of 0.645 reflecting only minor discriminatory ability. These criteria prioritize the inclusion of LN, resulting in a limited number of false negatives but a significant increase in false positives within SLE cases. Liu[35] referred to this trend is biologically feasible as circulating cfDNA serves as a general inflammatory marker that often increases during autoimmune activity, therefore intersecting with SLE symptoms. Recent evaluations

and data analyses indicate that total cfDNA levels are frequently increased in systemic lupus erythematosus (SLE) and correlate with disease activity metrics, hence restricting its disease-specific applicability when utilized in isolation.

The current study agrees with study done by Truszewska[36] who indicates elevated cfDNA levels in active systemic lupus erythematosus (SLE) and lupus nephritis (LN) relative to non-renal SLE or control groups, with connections observed with the SLE Disease Activity Index (SLEDAI) and anti-dsDNA antibodies. Discrepancies may arise from the inversion of reporting scales, the measurement of specific fractions, or pre-analytical processing. Research examining cfDNA size/integrity or epigenetic markers demonstrates superior differentiation of renal involvement, indicating that cfDNA profile is more significant than its concentration.

Furthermore, F. Wang referred to the cell-free DNA (cfDNA) as demonstrating potential as a biomarker in systemic lupus erythematosus (SLE), while its diagnostic efficacy is intricate. Research regularly shows increased cfDNA concentrations in SLE patients relative to healthy controls, with elevated levels linked to active lupus nephritis, which aligns with the present study (Table 9).

The test's sensitivity (74.3%) for identifying LN patients is notably high, indicating that cfDNA levels over the threshold are effective for recognising people with LN. Nevertheless, the low specificity (27.5%) suggests that the test inadequately differentiates LN from other illnesses, including SLE. This outcome aligns with the findings of F Wang[37], who indicated that cfDNA concentrations are markedly increased in individuals with SLE, particularly in those with severe manifestations of the disease. Patients with LN had markedly reduced cfDNA concentrations relative to those without LN, maybe linked to the existence of anti-double-stranded DNA antibodies[37].

A review by Giaglis[38] indicated that although cfDNA is sensitive to lupus nephritis, it lacks specificity in differentiating between lupus nephritis, other forms of SLE, and healthy individuals[38]. This outcome aligns with the present investigation, wherein the specificity was low, hence constraining the practical applicability of cfDNA as an independent diagnostic instrument.

The AUC of 0.461 in this study is comparatively low, signifying inadequate diagnostic accuracy for differentiating between lupus nephritis and SLE. This aligns with previous studies indicating that cfDNA alone may be inadequate for precise diagnosis and should be integrated with additional markers or clinical observations[39] (Table 10 and 11).

The study found a weak negative link between serum cell-free DNA (cfDNA) levels and disease severity in lupus nephritis (LN) patients, with a correlation coefficient of -0.021 and a p-value of 0.906. This conclusion corroborates prior studies suggesting that cfDNA levels in serum may not reliably indicate disease activity in autoimmune disorders such as systemic lupus erythematosus (SLE) and lupus nephritis (LN). Truszewska[30] found that higher levels of cfDNA were linked to active LN, but the fact that cfDNA profiles vary means that it may not be a good standalone biomarker for disease severity[33]. The study indicated a strong positive link between miRNA-192 fold change and disease severity in SLE patients, with a correlation coefficient of 0.041 and a p-value of 0.041, this results comes align with research done by Etemadi[40] that discovered increased concentrations of miRNA-192 in lupus nephritis (LN) patients, indicating its role in the pathogenesis of LN and its potential as a biomarker for disease activity[40].

The study also identified a non-significant positive link between urine cfDNA levels and illness severity, with a correlation coefficient of 0.126 and a p-value of 0.471. This outcome aligns with the research conducted by Aragón[41], which examined urinary biomarkers in LN and observed that although urinary cfDNA may serve as a possible marker[41] (Table 12).

CONCLUSION

Systemic lupus erythematosus is an autoimmune disturbance marked by poor augury and patients might experience several life-threatening complications particularly lupus nephritis, cardiovascular, and neural involvement. Therefore, there is a crucial demand for specific and accurate diagnostic tools and treatment targets to improve patients' lives. INF-Y levels were markedly increased in both SLE and LN patients relative to healthy controls, highlighting its involvement in the inflammatory etiology of both disorders.The expression of miRNA-27B was significantly reduced in the SLE and LN groups, demonstrating outstanding diagnostic accuracy (AUC = 1.00) in differentiating patients from healthy controls, underscoring its potential as a sensitive and specific biomarker. Urinary cfDNA concentrations were markedly elevated in SLE patients relative to those with LN, indicating that urine cfDNA may serve as a valuable diagnostic for distinguishing SLE from renal dysfunction. Serum levels of cell-free DNA were higher in patients, suggesting its significant role as a marker of systemic autoimmune activity and as a kidney-specific signal. These findings support the inclusion of cell-free DNA,

miRNA-27b, and INF-Y in comprehensive diagnostic and monitoring regimens for lupus nephritis.

REFERENCES

1. MJ, M., KZM, A., and MT, H. 2020, Association Between Systemic Lupus Disease Activity and Sleep Quality among Sample of Females with or without Depression at Baghdad Teaching Hospital. La Prensa Medica Argentina, 106: https://doi.org/10.47275/0032-745x-204
2. Connelly, K., and Morand, E. F. 2021, Systemic lupus erythematosus: a clinical update. Internal Medicine Journal, 51: 1219-1228. https://doi.org/10.1111/IMJ.15448;WGROUP:STRING:PUBLICATION
3. Moloi, M. W. 2019, Correlation of urinary mcp-1 and tweak with renal histology and early response to therapy in newly biopsied patients with lupus nephritis in cape town, south africa dissertation submitted in partial fulfillment of the requirements for award of the degree Master of Philosophy (MPhil) in Nephrology (Adult).
4. Roveta, A., Parodi, E. L., Brezzi, B., Tunesi, F., Zanetti, V., Merlotti, G., Francese, A., Maconi, A. G., and Quaglia, M. 2024, Lupus Nephritis from Pathogenesis to New Therapies: An Update. International Journal of Molecular Sciences 2024, 25: 8981, https://doi.org/10.3390/IJMS25168981
5. Guo, Q., Qiao, P., Wang, J., Zhao, L., Guo, Z., Li, X., Fan, X., Yu, C., and Zhang, L. 2024a, Investigating the value of urinary biomarkers in relation to lupus nephritis histopathology: present insights and future prospects. In Frontiers in Pharmacology 15). Frontiers Media SA. https://doi.org/10.3389/fphar.2024.1421657
6. Yung, S., and Chan, T. M. 2023, Endothelial cell activation and glycocalyx shedding - potential as biomarkers in patients with lupus nephritis. Frontiers in Immunology, 14. https://doi.org/10.3389/FIMMU.2023.1251876
7. Morales, E., Galindo, M., Trujillo, H., and Praga, M. (2021). Update on Lupus Nephritis: Looking for a New Vision. In Nephron (Vol. 145, Issue 1, pp. 1–13). S. Karger AG. https://doi.org/10.1159/000511268
8. So, B. Y. F., Yap, D. Y. H., and Chan, T. M. (2021). MicroRNAs in Lupus Nephritis–Role in Disease Pathogenesis and Clinical Applications. International Journal of Molecular Sciences 2021, 22:10737, https://doi.org/10.3390/IJMS221910737
9. Liang, S., Song, Z., Wu, Y., Gao, Y., Gao, M., Liu, F., Wang, F., and Zhang, Y. (2018). MicroRNA-27b Modulates Inflammatory Response and Apoptosis during Mycobacterium tuberculosis Infection. The Journal of Immunology, 200: 3506-3518. https://doi.org/10.4049/JIMMUNOL.1701448
10. Huang, B., Guo, F., Chen, J., Lu, L., Gao, S., Yang, C., Wu, H., Luo, W., and Pan, Q. (2025). Regulation of B-cell function by miRNAs impacting Systemic lupus erythematosus progression. Gene, 933, 149011. https://doi.org/10.1016/J.GENE.2024.149011
11. Burnham, P., Dadhania, D., Heyang, M., Chen, F., Westblade, L. F., Suthanthiran, M., Lee, J. R., and De Vlaminck, I. 2018, Urinary cell-free DNA is a versatile analyte for monitoring infections of the urinary tract. Nature Communications, 9. https://doi.org/10.1038/s41467-018-04745-0
12. Kirsch, C., Weickmann, S., Schmidt, B., and Fleischhacker, M. 2008, An improved method for the isolation of free-circulating plasma DNA and cell-free DNA from other body fluids. Annals of the New York Academy of Sciences, 1137: 135-139.
13. Gao, C., Jiao, Z., Wang, C., Dong, Y., Sun, and Xiao, J. (2018). Melon U6 gene and application thereof.
14. Bruno, S. M., Blaconà, G., Lo Cicero, S., Castelli, G., Virgulti, M., Testino, G., Pierandrei, S., Fuso, A., Cimino, G., Ferraguti, G., Eramo, A., and Lucarelli, M. (2023). Quantitative Evaluation of CFTR Gene Expression: A Comparison between Relative Quantification by Real-Time PCR and Absolute Quantification by Droplet Digital PCR. Genes, 14. https://doi.org/10.3390/genes14091781
15. Albrecht, K., Troll, W., Callhoff, J., Strangfeld, A., Ohrndorf, S., and Mucke, J. 2025, Sex- and gender-related differences in systemic lupus erythematosus: a scoping review. Rheumatology International, 45: 160. https://doi.org/10.1007/S00296-025-05910-7
16. Borrelli, R., Nicola, S., Corradi, F., Badiu, I., Lo Sardo, L., Rashidy, N., Quinternetto, A., Mazzola, M., Meli, F., Saracco, E., Vitali, I., Negrini, S., and Brussino, L. 2025. Impact of Sex on Infection Risk in Patients with Systemic Lupus Erythematosus. Bioengineering, 12:59. https://doi.org/10.3390/BIOENGINEERING12010059
17. Xiang, W., Xia, X., Lu, Y., Zhang, X., Shi, X., Jia, X., Tang, R., and Chen, W. (2024). Sex differences in mortality among patients with lupus nephritis. European Journal of Medical Research, 29: 500. https://doi.org/10.1186/S40001-024-02089-8/FIGURES/4
18. Vieira, A. A., Almada-Correia, I., Inácio, J., Costa-Reis, P., and da Rocha, S. T. 2024, Female-bias in systemic lupus erythematosus: How much is the X chromosome to blame? Biology of Sex Differences 15: 1-15. https://doi.org/10.1186/S13293-024-00650-Y
19. Khan, Z., Awais, M., Haq, W. U., Arshad, S., Aslam, M., Waheed, S., and Banaras, F. 2023, Prevalence of lupus nephritis in diagnosed patients of systemic lupus erythematosis (SLE) presenting to nephrology division, Khyber teaching hospital Peshawar. International Journal of Health Sciences (IJHS), 7: 409–414. https://doi.org/10.53730/IJHS.V7NS1.14215

20. Nyman, E., Vaughan, T., Desta, B., Wang, X., Barut, V., and Emmas, C. 2020. Characteristics and Symptom Severity of Patients Reporting Systemic Lupus Erythematosus in the PatientsLikeMe Online Health Community: A Retrospective Observational Study. Rheumatology and Therapy, 7: 201-213. https://doi.org/10.1007/S40744-020-00195-7
21. Vadhariya, A., Birt, J., Wu, J., Griffing, K., Bailey, F., Hetherington, J., Rottier, E., Barlow, S., Costenbader, K., Schilirò, D., Elefante, E., Stagnaro, C., Signorini, V., Zucchi, D., Trentin, F., Rocca, G. La, Carli, L., Ferro, F., Tani, C., and Mosca, M. 2022. Ab0554 clinical characteristics and burden among patients with sle stratified by sledai derived severity: results from a real-world study in the us. Annals of the Rheumatic Diseases, 81(Suppl 1), 1404.1-1404. https://doi.org/10.1136/ANNRHEUMDIS-2022-EULAR.4561
22. Katz, P., Andonian, B. J., and Huffman, K. M. 2020, Benefits and promotion of physical activity in rheumatoid arthritis. Current Opinion in Rheumatology, 32: 307-314. https://doi.org/10.1097/BOR.0000000000000696
23. Dörner, T., Tanaka, Y., Petri, M. A., Smolen, J. S., Wallace, D. J., Dow, E. R., Higgs, R. E., Rocha, G., Crowe, B., Benschop, R. J., Byers, N. L., Silk, M. E., De Bono, S., Fantini, D., and Hoffman, R. W. (2020). Baricitinib-associated changes in global gene expression during a 24-week phase II clinical systemic lupus erythematosus trial implicates a mechanism of action through multiple immune-related pathways. Lupus Science and Medicine, 7 https://doi.org/10.1136/LUPUS-2020-000424
24. Esmaeil Dorraji, S., Hovd, A. M. K., Kanapathippillai, P., Bakland, G., Eilertsen, G. Ø., Figenschau, S. L., and Fenton, K. A. 2018, Mesenchymal stem cells and T cells in the formation of Tertiary Lymphoid Structures in Lupus Nephritis. Scientific Reports 8: 1-16. https://doi.org/10.1038/s41598-018-26265-z
25. Yang, Y., Luo, R., Cheng, Y., Liu, T., Dai, W., Li, Y., Ge, S., and Xu, G. 2020, Leucine-rich a2-glycoprotein-1 upregulation in plasma and kidney of patients with lupus nephritis. BMC Nephrology, 21: 1-11. https://doi.org/10.1186/S12882-020-01782-0/FIGURES/5
26. Bai, L., Lin, Y., Xie, J., Zhang, Y., Wang, H., and Zheng, D. (2021a). MiR-27b-3p inhibits the progression of renal fibrosis via suppressing STAT1. Human Cell, 34: 383. https://doi.org/10.1007/S13577-020-00474-Z
27. Xu, Z. Q., Ding, Y., Huang, X. Y., Xiang, W., and He, X. J. 2021, CircELK4 Contributes to Lupus Nephritis by Acting as a miR-27b-3p Sponge to Regulate STING/IRF3/IFN-I Signaling. Inflammation, 44: 2106–2119. https://doi.org/10.1007/S10753-021-01487-Y,
28. Li, J., Ma, L., Yu, H., Yao, Y., Xu, Z., Lin, W., Wang, L., Wang, X., and Yang, H. 2022, MicroRNAs as Potential Biomarkers for the Diagnosis of Chronic Kidney Disease: A Systematic Review and Meta-Analysis. Frontiers in Medicine, 8: 782561. https://doi.org/10.3389/FMED.2021.782561/BIBTEX
29. Ibrahim, M. R. K., Waly, N. G., Moness, H., Ahmed, S. S., and Ibrahem, R. (2023). Serum miRNA-21, miRNA-146a and plasma cell free DNA as novel biomarkers for assessing systemic lupus erythematosus activity. Molecular Biology Reports, 50:, 10025. https://doi.org/10.1007/S11033-023-08845-Z
30. Truszewska, A., Wirkowska, A., Gala, K., Truszewski, P., Krzemien-Ojak, L., Perkowska-Ptasinska, A., Mucha, K., Paczek, L., and Foroncewicz, B. 2020a. Cell-free DNA profiling in patients with lupus nephritis. Lupus, 29: 1759-1772. https://doi.org/10.1177/0961203320957717,
31. Rafik, M. M., Amer, H. A., Zaitoun, Y., El Shnnawy, D., Lotfy, N., El azizi, N. O., and Youssef, M. A. (2024). Cell Free DNA in SLE Patients: A Possible Marker of Disease Activity and Organ Damage. QJM: An International Journal of Medicine, 117(Supplement_1). https://doi.org/10.1093/QJMED/HCAE070.155
32. Vodehnal, S., and Mohan, C. 2024. Urinary biomarkers for active Lupus Nephritis that have survived independent validation across cohorts. Kidney International, 106: 1135-1145. https://doi.org/10.1016/J.KINT.2024.09.007/ATTACHMENT/DC3F0BF1-BAAB-449E-9CAA-FBE726E092D6/MMC1.PDF
33. Truszewska, A., Wirkowska, A., Gala, K., Truszewski, P., Krzemien-Ojak, L., Perkowska-Ptasinska, A., Mucha, K., Paczek, L., and Foroncewicz, B. 2020b. Cell-free DNA profiling in patients with lupus nephritis. Lupus, 29: 1759-1772. https://doi.org/10.1177/0961203320957717,
34. Celec, P., Vlková, B., Lauková, L., Bábícková, J., and Boor, P. 2018. Cell-free DNA: the role in pathophysiology and as a biomarker in kidney diseases. Expert Reviews in Molecular Medicine, 20, e1. https://doi.org/10.1017/ERM.2017.12
35. Guo, Q., Qiao, P., Wang, J., Zhao, L., Guo, Z., Li, X., Fan, X., Yu, C., and Zhang, L. (2024b). Investigating the value of urinary biomarkers in relation to lupus nephritis histopathology: present insights and future prospects. Frontiers in Pharmacology, 15, 1421657. https://doi.org/10.3389/FPHAR.2024.1421657
36. Liu, F., Su, Y., Liu, X., Zhao, L., Wu, Z., Liu, Y., and Zhang, L. 2025. Cell-free DNA: a metabolic byproduct with diagnostic and prognostic potential in rheumatic disorders. Frontiers in Pharmacology,

16, 1537934. https://doi.org/10.3389/FPHAR.2025.1537934
37. Truszewska, A., Wirkowska, A., Gala, K., Truszewski, P., Krzemien-Ojak, L., Perkowska-Ptasinska, A., Mucha, K., Paczek, L., and Foroncewicz, B. (2020c). Cell-free DNA profiling in patients with lupus nephritis. Lupus, 29: 1759-1772. https://doi.org/10.1177/0961203320957717
38. Wang, F., Miao, H. B., Pei, Z. H., and Chen, Z. (2022). Serological, fragmentomic, and epigenetic characteristics of cell-free DNA in patients with lupus nephritis. Frontiers in Immunology, 13: 1001690. https://doi.org/10.3389/FIMMU.2022.1001690/FULL
39. Giaglis, S., Daoudlarian, D., Voll, R. E., Kyburz, D., Venhoff, N., and Walker, U. A. 2021, Circulating mitochondrial DNA copy numbers represent a sensitive marker for diagnosis and monitoring of disease activity in systemic lupus erythematosus. RMD Open, 7: https://doi.org/10.1136/RMDOPEN-2021-002010
40. Neuberger, E. W. I., Brahmer, A., Ehlert, T., Kluge, K., Philippi, K. F. A., Boedecker, S. C., Weinmann-Menke, J., and Simon, P. 2021. Validating quantitative PCR assays for cfDNA detection without DNA extraction in exercising SLE patients. Scientific Reports, 11: 1-11. https://doi.org/10.1038/S41598-021-92826-4;SUBJMETA
41. Etemadi, J., Jafari Nakhjavani, M. R., Motavalli, R., Mahmoodpoor, F., and Abediazar, S. 2019. Elevated levels of plasma microRNA-192 in patients with lupus nephritis. Immunopathologia Persa, 5: https://doi.org/10.15171/ipp.2019.02
42. Aragón, C. C., Tafúr, R. A., Suárez-Avellaneda, A., Martínez, M. T., Salas, A. de las, and Tobón, G. J. 2020. Urinary biomarkers in lupus nephritis. Journal of Translational Autoimmunity, 3: 100042. https://doi.org/10.1016/J.JTAUTO.2020.100042