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Value of sTNF-R1 and linc0597 as indicators for disease activity and diagnosis of lupus nephritis

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Abstract. – OBJECTIVE: To explore whether Soluble tumor necrosis factor-receptor 1 (sTNF-R1) and linc0597 can be used as indicators for disease activity and diagnosis of lupus nephritis (LN).

PATIENTS AND METHODS: Eighty LN patients treated in our hospital were enrolled as the LN group, while 60 Systemic Lupus Erythematosus (SLE) patients without nephritis were included in the SLE group, and 50 healthy subjects who conducted physical examination during the same period as the control group. After admission, 5 mL of venous blood was taken from all the study subjects to measure sTNF-R1 level and linc0597 expression by enzyme-linked immunosorbent assay (ELISA) and RT-qPCR respectively. In addition, the receiver operating characteristic (ROC) curves were employed to evaluate the diagnostic value of serum sTNF-R1 and linc0597 for LN, and Spearman correlation coefficient was adopted for the correlation between sTNF-R1, linc0597, and LN clinical disease Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). Moreover, the logistic multiple regression analysis was applied to analyze the independent risk factors affecting the complication of LN in SLE patients.

RESULTS: The LN group presented significantly higher serum sTNF-R1 and linc0597 levels than the control group and the SLE group. Besides, ROC curve analysis revealed that sTNF-R1 and linc0597 had good clinical diagnostic value in LN and SLE. Furthermore, Spearman correlation coefficient indicated that serum sTNF-R1 and linc0597 were positively correlated with disease activity index SLEDAI (r=0.551, $p<0.001$; R =0.604, $p<0.001$). Moreover, multivariate Logistic regression analysis demonstrated that age ($p=0.001$), fever ($p=0.004$), arthralgia (p=0.034), serum uric acid ($p=0.019$), decreased complement C3 (p=0.023), ANA peripheral type ($p=0.007$), anti-ds-DNA antibody ($p=0.003$), ANCA ($p=0.002$), sTNF-R1 ($p=0.001$), and linc0597 ($p<0.001$) were all independent risk factors affecting the complication of LN in SLE patients.

CONCLUSIONS: STNF-R1 and linc0597 can be used as the indicators for disease activity and diagnosis of LN.

Key Words:
LN, SLE, sTNF-R1, Linc0597, Risk factors.

Introduction

Systemic Lupus Erythematosus (SLE) is a chronic multi-system autoimmune disease associated with a high mortality rate. During the pathogenesis, multiple autoantibodies will be produced to form immune complexes, leading to multiple organ damage[1,2]. SLE complicated with renal dysfunction is called Lupus Nephritis (Lupus Nephritis, LN), once appear, progressive renal damage is likely to worsen the condition of patients with SLE, with LN being a major cause of death[3,4]. According to statistics, up to 30-50% of SLE patients will develop LN, while SLE patients complicated with LN have an increased risk of developing end-stage renal disease[5]. Therefore, exploring biomarkers is a must for better diagnosis due to the heterogeneity of LN patients and their unpredictable course of disease.

Tumor necrosis factor (TNF) refers to a key endogenous cytokine and inflammatory mediator in regulating human immune response, which involves the repair and reconstruction of tissue injury, and TNF is reported to exert regulatory effect on renal diseases[6]. All the biological functions of TNF-α are mediated by soluble tumor necrosis factor-receptor 1 (sTNF-R1) on target cells. As to sTNF-R1, it is a receptor mainly expressed on cells sensitive to cytotoxicity of TNF-α and on the surface of activated T and B lymphocytes. Since sTNF-R1 in blood circulation mainly comes from activated lymphocytes, it can be inferred that lymphocytes have been activated by antigen by measuring sTNF-R1, thus indirectly determining disease activity[7]. Long non-coding RNA (lncRNA) is a non-coding protein RNA with at least 200 nucleotides implicated

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in immune regulation, which has the function of regulating the growth and differentiation of immune cells, such as T cells, B cells and NK cells, and is related to the occurrence and development of human gastric cancer, cardiovascular diseases and various autoimmune diseases[8-10]. Long intergenic noncoding RNA (lincRNA), a type of lncRNA that represents the longest group of non-coding RNAs, is named for its location in the region between the two genes in the genome and adjacent protein-coding genes, which has been confirmed to be related to the pathological mechanism of SLE[11].

In this study, the expression levels of sTNF-R1 and linc0597 in the serum of different groups were detected to compare their diagnostic efficacy in the three groups, as well as to analyze the their correlation in the disease activity of LN, so as to verify whether sTNF-R1 and linc0597 can serve as the indicators for the disease activity and diagnosis of LN.

Patients and Methods

Patients

Eighty LN patients admitted to our hospital were selected as the LN group, including 35 males and 45 females, aged 20-79 years, with an average age of (27.2±8.3). Another 60 SLE patients were enrolled as the SLE group, including 20 males and 40 females, aged 21-78 years, with an average age of (32.1±7.5) years. In addition, 50 healthy subjects were assigned into the control group, including 20 males and 30 females, aged 19-79 years, with an average age of (33.7±10.6) years. The guardians of the study subjects all understood the situation of the study and signed the informed consent. This study goes along with ethics and morality, whose proposal is submitted to the Ethics Committee of Traditional Chinese Hospital of LuAn for review and approval before implementation.

Inclusion and Exclusion Criteria

Inclusion criteria. Patients aged over 18 years old, without history of psychosis, nor use of uric-acid-lowering drug, kidney preserving drugs, diuretics or other drugs before admission, and those met the SLE diagnostic criteria established by 1997 American College of Rheumatology[12] and the criteria for kidney damage stated as below: urinary protein >0.5 g/d or tubular urine; red blood cells, mixed tubular or hemoglobin, granules (a must in the LN group, while not required in the SLE group). Written informed consent was obtained from all patients or their families.

Exclusion criteria. Patients with cardiovascular diseases, acute infections, autoimmune diseases, other malignant tumors, acute or chronic infectious diseases; patients with other secondary or primary renal diseases; patients in pregnancy or lactation; patients with recent infection, recent immunosuppressant or high dose hormone therapy.

The inclusion criteria mentioned above were applicable to the LN group and the SLE group, and the healthy subjects were assigned into the control group.

Detection of Serum sTNF-R1 Level

An amount of 5 mL of elbow venous blood was extracted from subjects within 24 h after admission and placed in vacuum blood collection vessel without anticoagulant. Followed by the centrifugation of 1500 g for 15 min. Then, the serum was retained and stored in Eppendorf (EP; Hamburg, Germany) tube and stored in low-temperature refrigerator at -80°C for later use. Next, the serum was removed from the freezer and dissolved in a 4°C refrigerator, and then, completely dissolved at room temperature. Enzyme-linked immunosorbent assay (ELISA)[13] was employed to detect the serum sTNF-R1 level, with reference to the manual of human sTNF-R1 ELISA kit (Tecan Trading Co., Ltd., Shanghai, China, Article No.: BE45701). The standard well and the blank well were set up, among which the sample wells were added with 50 μL of the sample to be tested, the standard wells with 50 μL of the standard, while the blank wells remained as they were. After that, 100 μL of the horseradish peroxidase-labeled detection antibody was added to the sample well and the standard well, and the plate was sealed and incubated at 37°C for 60 min. The liquid was then discarded, dried, and washed repeatedly 5 times. Next, the substrates A and B were thoroughly mixed in at a ratio of 1:1, followed by adding 100 μL of the liquid to the wells, and then, we sealed the plate and incubated at 37°C for 15 min. Finally, 50 μL of termination solution was added to each well, and the absorbance (OD) of each well at 450 nm was read by MB-530 full-automatic enzyme label analyzer (Chenlian Biotechnology Development Co., Ltd., Shanghai, China). The reference wavelength of sTNF-R1 was 620 nm, and the sTNF-R1 level was calculated.

RT-qPCR for the Detection of Linc0597 Expression

The total RNA was extracted from the serum by TRIzol extraction kit (Huiying Biological Technology Co., Ltd., Shanghai, China: Article No.: 15596026), the RNA concentration and purity were detected by DR5000 ultraviolet spectrophotometer (Xizhenlongbo Science and Technology Co., Ltd., Beijing, China), and the integrity of RNA by 1% agarose gel electrophoresis. RNA was reversely transcribed into cDNA according to the instructions of the reverse transcription kit (Kanglang Biological Technology Co., Ltd., Shanghai, China; KL033), and the samples after reverse transcription were stored at -20°C for later use. With GAPDH as the internal reference, the upstream and downstream of linc0597 and GAPDH were as below: Linc0597 upstream: 5'-TTGGATTCATCCCGTTCACCTCCA-3', Linc0597 downstream: 5'-CAGCATGACGATCAAGCGAGATTC-3'. GAPDH upstream: 5'-GGGAAACTGTGGCGTGAT-3', GAPDH downstream: 5'-GAGTGGGTGTCGCTGTTGA-3'. The reaction was performed with the CFX384 Touch Real-Time Fluorescence quantitative PCR instrument (Guangyao Medical Equipment Co., LTD., Jinan, China). The PCR cyclic amplification conditions were as follows: pre-denaturation at 95°C for 30s, denaturation at 95°C for 5s, annealing extension at 60°C for 30 s, totaling for 40 cycles. The data obtained after three independent experiments were taken, and the relative gene expression was expressed through $2^{-\Delta\Delta CT}$.

Statistical Analysis

The collected data were statistically analyzed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA) and plotted by GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). The measurement data were represented by mean± standard deviation (mean±SD). Among which, the inter-group comparison was performed by a *t*-test of independent samples, while One-way ANOVA was adopted for the comparison among the three groups, and the post-hoc pairwise comparison of the mean was then performed using the Bonferroni. The counting data were expressed as case/percentage [n(%)] and a Chi-square test was employed for inter-group comparison. Receiver operating characteristic (ROC) curves were applied to evaluate the diagnostic value of serum sTNF-R1 and linc0597 in the three groups, as well as to determine the diagnostic efficacy of combined detection of sTNF-R1 and linc0597 for LN. The Spearman correlation coefficient analysis was conducted to assess the correlation between sTNF-R1, linc0597, and LN clinical disease Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)[14]. Independent risk factors affecting the complication of LN in SLE patients were analyzed by Logistic multivariate regression. A statistically significant difference was assumed at $p<0.05$.

Results

Analysis of General Data of Subjects in the Three Groups

Except the notably difference in age ($p<0.05$), no other significant differences were observed in sex, course of disease, BMI, dietary preference, smoking history, educational level, alcohol abuse, and other general data ($p>0.05$) among the three groups (Table I).

Comparison of Serum Levels of sTNF-R1 and Linc0597

The serum sTNF-R1 levels in the control group, LN group, and SLE group were (2.05±0.52) ng/ml, (3.71±1.12) ng/ml, (2.57±1.01) ng/ml respectively, and the corresponding relative expression levels of serum linc0597 were (0.73±0.44), (1.99±0.89), and (1.58±0.59). It was evident that the serum sTNF-R1 and linc0597 levels in the LN group were significantly higher than those in the rest of the two groups ($p<0.001$) (Figure 1).

Diagnostic Value of Serum sTNF-R1 and Linc0597 in Different Groups

ROC curve analysis indicated that sTNF-R1 and linc0597 were of good clinical diagnostic value in LN and SLE. The first comparison went to the control group and LN group. The AUC value of serum sTNF-R1 of the two groups was 0.844, and the optimal cut-off value was 2.70 ng/ml. Their corresponding AUC value and optimal cut-off value of serum linc0597 were 0.886, and 1.35; while the AUC value of combined diagnosis of serum sTNF-R1 and linc0597 was 0.945, with the optimal cut-off value of 0.77. Regarding the control group and SLE group, the AUC value of serum sTNF-R1 of the two groups was 0.781, and the optimal cut-off value was 2.78 ng/ml. Their AUC value and optimal cut-off value of serum linc0597

Table I. Analysis of general data of subjects in the three groups.

Indicators	The control group	The LN group	The SLE group	χ^2/F/t	p
China				82.078	3298
Gender				1.565	0.457
Male	20 (40.00)	35 (43.75)	20 (33.33)		
Female	30 (60.00)	45 (56.25)	40 (66.67)		
Age (years)	33.7 ± 10.6	27.2 ± 8.3	32.1 ± 7.5	10.07	< 0.05
Course of disease (month)	–	13.35 ± 2.88	13.27 ± 2.74	0.166	0.868
BMI (kg/m^2)	23.98 ± 2.73	24.11 ± 2.68	24.05 ± 2.61	0.037	0.964
Dietary preference				1.609	0.447
Light	26 (52.00)	41 (51.25)	25 (41.67)		
Spicy	24 (48.00)	39 (48.75)	35 (58.33)		
Smoking history				3.466	0.177
Yes	21 (42.00)	47 (58.75)	31 (51.67)		
No	29 (58.00)	33 (41.25)	29 (48.33)		
Educational level				4.005	0.135
≥ high school	31 (62.00)	36 (45.00)	34 (56.67)		
<high school	19 (38.00)	44 (55.00)	26 (43.33)		
Alcohol abuse				4.898	0.086
Yes	17 (34.00)	43 (53.75)	29 (48.33)		
No	33 (66.00)	37 (46.25)	31 (51.67)		

were 0.849 and 0.90, respectively; while the AUC value of combined diagnosis of serum sTNF-R1 and linc0597 was 0.891, with the optimal cut-off value of 0.46. The last comparison were between the LN group and the SLE group, whose AUC value and optimal cut-off value of serum sTNF-R1 were 0.778 and 2.39 ng/ml respectively, and the corresponding serum linc0597 AUC value and the optimal cut-off value were 0.773 and 1.90, while the combined diagnosis of AUC value of serum sTNF-R1 and linc0597 was 0.845, with the optimal cut-off value of 0.40 (Table II, Figure 2).

Correlation of Serum sTNF-R1 and Linc0597 Levels with Disease Activity Index SLEDAI in the LN Group

SLEDAI was used to evaluate the disease activity degree of LN patients, whose score and evaluation were as follows: 0-4 points for inactive, 5-9 points for mild, 10-14 points for moderate, and >14 points for severe. Spearman correlation coefficient showed that the serum sTNF-R1 and linc0597 were positively correlated with the disease activity index SLEDAI (r=0.551, p<0.001; R =0.604, p<0.001) (Figure 3).

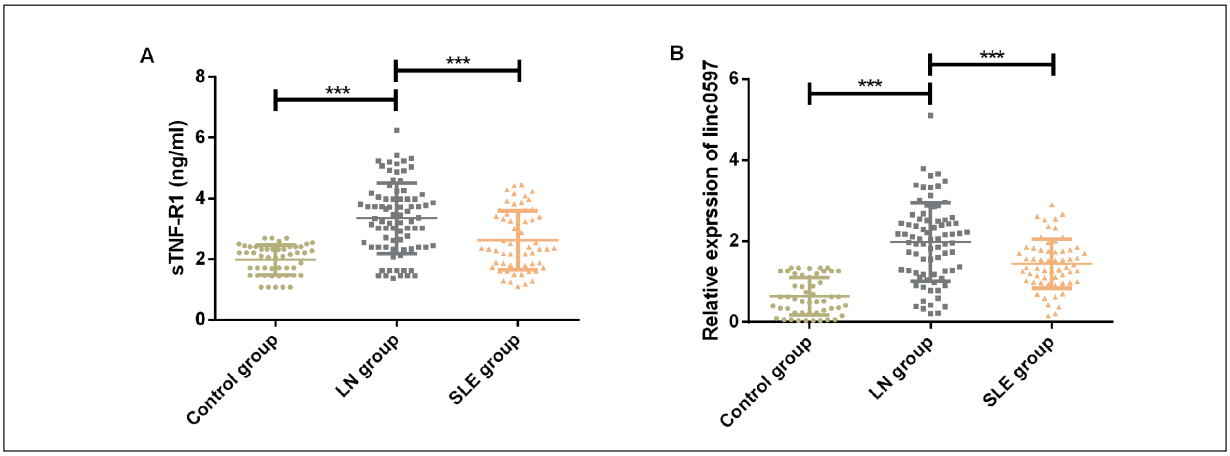

Figure 1. Comparison of serum levels of sTNF-R1 and linc0597. Serum levels of sTNF-R1 (**A**) and linc0597 (**B**) in the LN group were significantly higher than those in the control group and SLE group (p<0.001). Note: ***Indicated p<0.001.

Table II. ROC parameters.

Groups	Indicators	AUC	95% CI	Standard error	Cut-off value	Sensitivity (%)	Specificity (%)
The control group and the LN group	sTNF-R1	0.844	0.776-0.912	0.034	2.70 ng/ml	72.50	82.00
	linc0597	0.886	0.832-0.941	0.028	1.35	70.00	84.00
	sTNF-R1+llinc0597	0.945	0.908-0.983	0.019	0.77	81.25	94.00
The control group and the SLE group	sTNF-R1	0.781	0.582-0.780	0.050	2.78 ng/ml	70.00	70.00
	linc0597	0.849	0.780-0.918	0.035	0.90	86.67	70.00
	sTNF-R1+llinc0597	0.891	0.833-0.949	0.030	0.46	88.33	76.00
The LN group and the SLE group	sTNF-R1	0.778	0.590-0.767	0.045	2.39 ng/ml	63.33	80.00
	linc0597	0.773	0.583-0.762	0.046	1.90	81.67	67.50
	sTNF-R1+llinc0597	0.845	0.664-0.825	0.041	0.40	83.33	70.00

Analysis of Clinical Symptoms and Pathological Parameters in the LN Group and SLE Group

The comparison of clinical symptoms and pathological parameters represented by butterfly blush, serum creatinine, leukopenia, anemia, erythrocytopenia, decreased complement C4, ANA, ANA spot, and nucleolus types did not identify any significant differences ($p>0.05$) between the LN group and SLE group ($p>0.05$). While there were significant differences in fever, arthralgia, serum uric acid, thrombocytopenia, decreased complement C3, ANA homogeneous and peripheral types, anti-ds-DNA, anti-sms, ANCA, sTNF-R1 and linc0597 ($p<0.05$) (Table III).

Logistic Multivariate Regression Analysis for Risk Factors Affecting the Complication of LN in SLE Patients

The optimal critical values of sTNF-R1 (2.39 ng/ml) and linc0597 (1.90) for diagnosing LN and SLE were set, as the segmentation points to compare the differences of general data, clinical symptoms and pathological parameters between the LN group and SLE group. The significantly differentiated factors represented by age, fever, arthralgia, serum uric acid, thrombocytopenia, decreased complement C3, ANA homogeneous type, ANA peripheral type, anti-ds-DNA antibody, anti-sm antibody, ANCA, sTNF-R1, linc0597 between the LN and SLE groups ($p<0.05$) were analyzed by multivariate Logistic regression analysis. The results revealed that age ($p=0.001$), fever ($p=0.004$), arthralgia ($p=0.034$), serum uric acid ($p=0.019$), decreased complement C3 ($p=0.023$), ANA peripheral type ($p=0.007$), anti-ds-DNA antibody ($p=0.003$), ANCA ($p=0.002$), sTNF-R1 ($p=0.001$), and linc0597 ($p<0.001$) were independent risk factors affecting the complication of LN in SLE patients. While patients with SLE younger than 30 years old, who presented with fever, arthralgia, high serum uric acid (>0.50mmol/L), decreased complement C3, positive ANA peripheral type, positive anti-ds-DNA antibody, positive ANCA, high sTNF-R1 (>2.39), and high linc0597 (>1.90 ng/ml) expression were at increased risk of developing LN (Table IV and Table V).

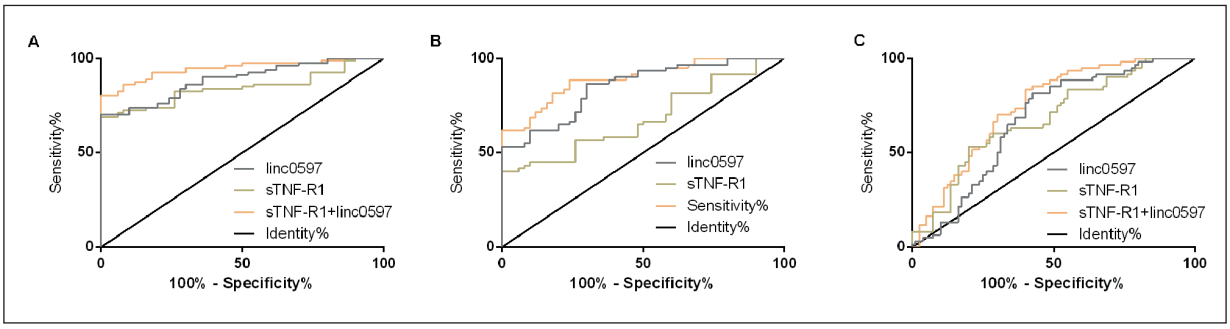

Figure 2. ROC curves for the diagnostic value of serum sTNF-R1 and linc0597. **A,** Diagnostic value of serum sTNF-R1 and linc0597 in the control group and LN group. **B,** Diagnostic value of serum sTNF-R1 and linc0597 in the control group and SLE group. **C,** Diagnostic value of serum sTNF-R1 and linc0597 in the LN group and SLE group.

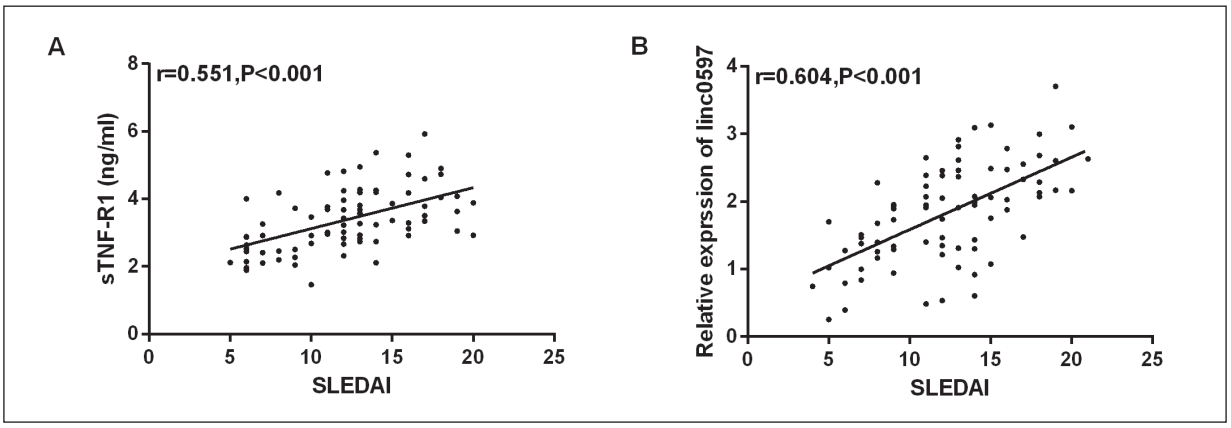

Figure 3. Serum sTNF-R1 (**A**) and linc0597 (**B**) in the LN group were positively correlated with disease activity index SLEDAI. Serum sTNF-R1 (**A**) and linc0597 (**B**) were positively correlated with SLEDAI (r=0.551, $p<0.001$; R =0.604, $p<0.001$).

Discussion

LN is one of the most common complications of SLE, with an incidence of up to 50% and approximately 20% progressing to end-stage renal disease (ESRD), depending on the study population[15,16]. Although recent years have witnessed the rapid progress in medical diagnosis and therapy, the treatment of LN patients is still not ideal, and the incidence of LN patients shows an upward trend[17]. Characterized by low overall biopsy rate and high injury, renal pathological biopsy, however, remains the gold standard for diagnosis of LN today[18]. Du et al[19] have identified the biomarkers associated with LN disease activity or prognosis, such as serum Semaphorin5A. Whereas, a more sensitive, specific and ideal non-invasive biomarker is still under investigation for diagnosing LN and monitoring disease activity. Therefore, a novel biomarker that can more effectively diagnose LN and predict disease needs to be further explored.

It is well established that sTNF-R1, a pro-inflammatory tumor necrosis factor-α (TNF-α) receptor involved in immune regulation, has been shown to have the potential to diagnose LN. For example, Wu et al[20] found that the levels of urine VCAM-1, P-selectin, sTNF-R1, and CXCL-16 levels were elevated in multiple strains of LN mice and LN patients, and all the four molecules showed excellent ROC curves in urine, with the AUC in diagnosing SLE of 0.76 to 0.89, presenting high sensitivity and specificity for differentiating SLE patients and healthy control groups. In recent years, the research focus of sTNF-R1 in LN has shifted from adults to adolescents. Patel et al[21] reported that juvenile systemic lupus erythematosus (JSLE) is a debilitating condition commonly seen in LN. By measuring the plasma levels of sTNF-R1 in JSLE patients and the healthy control group (HC), it was found that sTNF-R1 significantly increased in JSLE patients compared with the HC group, but significantly increased in active LN compared with inactive LN. These studies suggest that sTNF-R1 may, to some extent, reflect the disease activity of LN. In this study, the serum sTNF-R1 levels in the LN group were markedly higher than those in the control group and SLE group ($p<0.001$). In addition, ROC curve analysis demonstrated that the AUC values of serum sTNF-R1 in the control group and LN group, the control group and SLE group, and the LN group and SLE group were 0.844, 0.781, and 0.778, respectively. Furthermore, the disease correlation analysis revealed that the serum sTNF-R1 in the LN group was positively correlated with the disease activity index SLEDAI. The results showed that the serum sTNF-R1 alone was of general value in the control group, LN group, and SLE group, and had certain value in assessing the disease activity of LN patients.

LincRNA is a large, long non-coding RNA of the lncRNA family, which has been constantly proved to be bound up with inflammation and even cancer. According to some studies, the knockdown of LincRNA-Gm4419 gene expression can improve the inflammatory response mediated by NF-κB/NLRP3 inflammatory body in diabetic nephropathy. It is also reported[22,23] that the overexpression of long intergenic non-coding RNA HOTAIR regulates PTEN methylation in

Table III. Comparison of clinical symptoms and pathological parameters between the LN group and SLE group ([n (%)], ($\bar{x} \pm sd$).

Indicators	The LN group (n = 80)	The SLE group (n = 60)	χ^2/t	p
Fever			18.86	< 0.001
Yes	42 (52.50)	10 (16.67)		
No	38 (47.50)	50 (83.33)		
Butterfly blush			0.906	0.341
Yes	22 (27.50)	21 (35.00)		
No	58 (72.50)	39 (65.00)		
Arthralgia			9.880	< 0.05
Yes	38 (47.50)	13 (21.67)		
No	42 (52.50)	47 (78.33)		
Serum uric acid (mmol/L)	0.55 ± 0.16	0.43 ± 0.13	4.750	< 0.001
Serum creatinine (mg/dL)	0.72 ± 0.32	0.68 ± 0.29	0.762	0.448
Leukopenia			1.094	0.296
Positive	35 (43.75)	21 (35.00)		
Negative	45 (56.25)	39 (65.00)		
Anemia			0.063	0.801
Yes	49 (61.25)	38 (63.33)		
No	31 (38.75)	22 (36.67)		
Erythrocytopenia			1.225	0.228
Positive	24 (30.00)	13 (21.67)		
Negative	56 (70.00)	47 (78.33)		
Thrombocytopenia			3.444	< 0.001
Positive	37 (46.25)	11 (18.33)		
Negative	43 (53.75)	49 (81.67)		
Decreased complement C3			10.45	< 0.05
Positive	46 (57.50)	18 (30.00)		
Negative	34 (42.50)	42 (70.00)		
Decreased complement C4			1.612	0.204
Positive	42 (52.50)	25 (41.67)		
Negative	38 (47.50)	35 (58.33)		
ANA			0.064	0.801
Positive	73 (91.25)	54 (90.00)		
Negative	7 (8.75)	6 (10.00)		
ANA homogeneous type			11.212	<0.001
Positive	14 (17.50)	26 (43.33)		
Negative	66 (82.50)	34 (56.67)		
ANA peripheral type			9.989	< 0.01
Positive	20 (33.33)	3 (5.00)		
Negative	60 (66.67)	57 (95.00)		
ANA spot type			0.088	0.766
Positive	34 (42.50)	24 (40.00)		
Negative	46 (57.50)	36 (60.00)		
ANA nucleolar type			0.206	0.650
Positive	7 (8.75)	4 (6.67)		
Negative	73 (91.25)	56 (93.33)		
Anti-ds-DNA antibody			6.905	< 0.001
Positive	62 (77.50)	34 (56.67)		
Negative	18 (22.50)	26 (43.33)		
Anti SM antibody			8.091	< 0.001
Positive	22 (27.50)	5 (8.33)		
Negative	58 (72.50)	55 (91.67)		
ANCA			8.260	< 0.001
Positive	26 (32.50)	7 (11.67)		
Negative	54 (67.50)	53 (88.33)		
sTNF-R1 (ng/ml)	3.71 ± 1.12	2.57 ± 1.01	6.213	< 0.001
linc0597	1.99 ± 0.89	1.48 ± 0.59	3.848	< 0.001

laryngeal squamous cell carcinoma. In addition, lincRNA has been widely reported in a variety of autoimmune diseases, which regulates congenital and adaptive immune response, immune cell development, and autoimmune diseases through differential expression levels, including systemic

Table IV. Logistic multivariate regression analysis assignments affecting LN in patients with SLE.

Factors	Variables	Assignments
Age (years)	X1	≤ 30 = 1, > 30 = 2
Fever	X2	Yes = 1, no = 2
Arthralgia	X3	Yes = 1, no = 2
Serum uric acid (mmol/L)	X4	> 0.50 = 1, ≤ 0.50 = 2
Thrombocytopenia	X5	Positive = 1, negative = 2
Decreased complement C3	X6	Positive = 1, negative = 2
ANA homogeneous type	X7	Positive = 1, negative = 2
ANA peripheral type	X8	Positive = 1, negative = 2
Anti-ds-DNA antibody	X9	Positive = 1, negative = 2
Anti SM antibody	X10	Positive = 1, negative = 2
ANCA	X11	Positive = 1, negative = 2
sTNF-R1 (ng/ml)	X12	> 2.39 = 1, ≤ 2.39 = 2
linc0597	X13	> 1.90 = 1, ≤ 1.90 = 2

lupus erythematosus (SLE), type 1 diabetes mellitus (T1DM), and autoimmune thyroid disease (AITD)[24-26]. MicroRNA has been the hotspot in the LN molecular mechanism related research in recent years, while little has been done on linc0597 except exploring its expression and diagnostic efficacy in SLE. Of note, in the study of Wu et al[27], the plasma linc0597 was upregulated in SLE and LN patients, suggesting that plasma linc0597 might be a potential biomarker of SLE and LN, and was associated with the occurrence and development of the two diseases. In the present study, the serum linc0597 level in the LN group were significantly higher than that in the control group and SLE group ($p<0.001$). In addition, ROC curve analysis demonstrated that the AUC values of serum linc0597 alone in the control group and LN group, the control group and SLE group, and the LN group and SLE group were 0.886, 0.849, and 0.773, respectively. Furthermore, disease correlation analysis revealed that the serum linc0597 in the LN group was positively correlated with disease activity index SLEDAI. The results showed that, compared with the single detection with serum sTNF-R1, the serum linc0597 was of better value in the control group, LN group, and SLE group, and had better potential for assessing the disease activity of patients with LN.

We investigated the diagnostic efficacy of the combined serum sTNF-R1 and linc0597 in the three groups. ROC curve analysis showed that the AUC value of combined detection of the two in the control group and LN group, the control group and SLE group, and the LN group and SLE group were 0.945, 0.891, 0.845, respectively. These results indicates that the combined diagnostic efficacy is better than that of single detection, and possesses the best efficacy for diagnosing and differentiating the control group and LN group, suggesting that the combined detection of serum sTNF-R1 and linc0597 has good diagnostic value for the identification of the three groups, and that the two may become new minimally invasive biomarkers for patients with LN. Furthermore, we explored the risk factors

Table V. Multivariate Logistic regression analysis for determination of risk factors for SLE patients with LN.

Variables	B	SLE	Wals	p	OR	95% CI
Age	0.550	0.865	2.284	0.001	1.778	1.014-2.829
Fever	1.026	0.456	7.071	0.004	4.791	1.142-19.823
Arthralgia	1.354	0.701	5.792	0.034	3.916	1.282-13.512
Serum uric acid (mmol/L)	1.987	0.694	5.292	0.019	2.848	1.251-7.005
Decreased complement C3	1.116	0.491	5.339	0.023	3.075	1.178-7.698
ANA peripheral type	2.101	0.790	7.025	0.007	8.002	1.733-37.095
Anti-ds-DNA antibody	2.179	0.761	8.419	0.003	8.812	2.027-38.711
ANCA	1.392	0.605	5.953	0.002	6.692	2.015-31.79
sTNF-RI (ng/ml)	2.593	0.376	10.986	0.001	14.785	3.129-68.837
linc0597	3.316	0.229	12.305	0.000	15.293	1.866-98.357

for the development of LN in SLE patients and found that patients with SLE younger than 30 years old, who presented with fever, arthralgia, high serum uric acid (>0.50 mmol/L), decreased complement C3, positive ANA peripheral type, positive anti-ds-DNA antibody, positive ANCA, high sTNF-R1 (>2.39), and high linc0597 (>1.90 ng/ml) expression were at increased risk of developing LN, with the highest risk multiples of high sTNF-R1(>2.39) and high linc0597 (>1.90) expression.

We verified that sTNF-R1 and linc0597 have certain diagnostic value for LN and are positively correlated with the disease activity of LN, but there is still room for improvement. First of all, we hope that basic experiments can be carried out in the future to establish animal models and supplement the discussion of specific regulation mechanism. Secondly, the sample size should be increased to include teenagers and the elderly, so as to improve the significance of experimental results. What's more, more precise testing instruments are to be selected to improve the experimental accuracy. Moreover, the expression levels and diagnostic potential of sTNF-R1 and linc0597 in LN patients can be compared from the perspectives of urine and saliva to explore the relationship between urine and saliva diagnosis and the inflammatory process of autoimmune diseases.

Conclusions

In short, sTNF-R1 and linc0597 can serve as criteria for disease activity and diagnosis of LN.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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