Efficacy and Prognostic Prediction of TL1A and Serum Cystatin C on Prednisone Combined with Leflunomide for Lupus Nephritis

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Objective：This study set out to investigate the efficacy and prognostic prediction of TL1A and serum cystatin C in the treatment of lupus nephritis (LN) with prednisone and leflunomide.

Methods: Sixty-four LN patients treated in our hospital from June 2020 to March 2022 were selected as a research group, and all patients were treated with prednisone combined with leflunomide. Sixty-four healthy people who underwent physical examination in our hospital during the same period were selected as a control group. Serum cystatin C and tumor necrosis factor-like ligand 1A (TL1A) were detected before and after treatment in patients from both groups, and their predictive values for the efficacy and prognosis of LN patients were analyzed.

Results: Before treatment, the expression levels of serum cystatin C and TL1A in the research group were significantly higher than those in the control group ($P<0.05$); after treatment, the expression levels of serum cystatin C and TL1A in the research group were significantly lower than those before treatment, but still higher than those in normal people ($P<0.05$). After treatment, the 24-hour urine protein quantitation, Scr, and BUN in the research group were significantly lower than those before treatment, while the plasma albumin and C3 were significantly higher than those before treatment ($P<0.05$). The expression levels of cystatin C and TL1A in serum of patients in the effective group were significantly lower than those in the ineffective group before treatment ($P<0.05$). ROC analysis found that the predicted AUC of cystatin C for ineffective treatment was 0.917, and the predicted AUC of TL1A for ineffective treatment was 0.860. The expression levels of cystatin C and TL1A in serum of patients with good prognosis after treatment were significantly lower than those with poor

prognosis (P<0.05). ROC analysis found that serum cystatin C had a predicted AUC of 0.855 for patients with LN and TL1A had a predicted AUC of 0.850 for patients with LN. Multivariate analysis showed that the increase of 24-hour proteinuria, serum cystatin C and TL1A, as well as renal pathological grading were independent risk factors for poor prognosis of LN patients.

Conclusion: serum cystatin C and TL1A had high predictive value for the efficacy and prognosis of prednisone combined with leflunomide in the treatment of lupus nephritis, and the increase of serum cystatin C and TL1A were independent predictive factors for poor prognosis of LN patients, which might provide a certain reference direction for their treatment and prognosis.

Keywords: TL1A, cystatin C, prednisone, leflunomide, lupus nephritis, efficacy, prognostic prediction

INTRODUCTION

Lupus nephritis (LN), as a secondary immune complex nephritis, is mainly due to kidney injury caused by kidney invasion by systemic lupus erythematosus as an autoimmune disease [1]. LN is mainly characterized by abnormal renal function, and glomerulonephritis caused by LN has a more complicated pathogenesis. It's more difficult to treat it clinically, and its course of disease is longer, with poor prognosis and high possibility of relapse [2,3]. Therefore, it is of great clinical significance for LN patients to effectively treat LN and predict its efficacy. At present, the clinical treatment of LN mainly adopts the combination of glucocorticoid and immunosuppressant. Although it has good efficacy for most patients, there are still some patients whose efficacy is not ideal. If the treatment scheme cannot be adjusted in time, the prognosis of them will be adversely affected [4,5].

Prednisone, as a glucocorticoid, has anti-inflammatory and anti-allergic effects. It can effectively stabilize lysosomal membrane and reduce fibrin deposition, thus decreasing proteinuria and achieving efficacy by reducing capillary permeability. At present, prednisone is also widely used in clinical practice [6]. Leflunomide, as a low

toxicity immunosuppressant, not only can inhibit cell proliferation and excessive immune response, but also can improve renal interstitial fibrosis with fewer adverse reactions [7]. Although prednisone and leflunomide are both widely used clinically, there is still some controversy about the efficacy of their combined application. At present, clinical evaluation of the efficacy of LN is mostly carried out by creatinine or urea nitrogen, but the changes of these indexes are not obvious for patients with minor renal injury, and cannot reflect the condition or efficacy of patients very well [8]. serum cystatin C is a protein constantly produced by nucleated cells in the body. Because it can only be filtered through glomerulus, it can be used as an important marker for evaluating renal function and efficacy of drugs [9]. Tumor necrosis factor-like ligand 1A (TL1A), as a member of tumor necrosis factor superfamily, was thought to form TL1A-DR3 complexes by binding with 3 (death receptor 3, DR3) of its receptor superfamily members, thus stimulating T cell activation and further promoting inflammatory response [10]. Previous studies [11] have shown that TL1A plays an important role in various autoimmune diseases, but few studies have analyzed its expression and clinical significance in LN.

In order to find a new effective scheme for the treatment of LN, we investigated the efficacy of prednisone combined with leflunomide on LN and effect on the expression levels of serum cystatin C and urine N-acetyl-β-D-glucosidase.

1. MATERIALS AND METHODS

1.1 General information

Sixty-four LN patients treated in our hospital from June 2020 to March 2022 were selected as the research group, including 34 male patients and 30 female patients; the average age of all patients was (37.21±6.33) years old, and they were treated with prednisone combined with leflunomide. Sixty-four healthy people who underwent physical examination in our hospital during the same period were selected as the control group. Inclusion and exclusion criteria were as follows: patients diagnosed as LN by pathological diagnosis. Exclusion criteria: patients with primary nephritis;

patients who had taken hormone or immunosuppressant orally in the past 2 months; patients with severe cardiopulmonary insufficiency; patients with communication or cognitive impairment; patients who did not cooperate with this study. All patients and their families agreed to participate in the experiment and sign an informed consent. The experiment was approved by the Hospital Ethics Committee.

1.2 Treatment methods

All patients in the research group took prednisone tablets， with an initial dose of 10mg/(kg·d). After the condition of patients improved, the dosage was reduced to a maintenance dose of 10mg/d according to their specific conditions. Then 40mg/d leflunomide on the basis of prednisone was taken orally, and it was reduced to 20mg/d after 3 days, with a treatment period of 6 months. After efficacy evaluation, prednisone combined with cyclophosphamide was used for the treatment of patients with ineffective treatment.

1.3 Index detection

Altogether 5ml of venous blood was taken from patients in the research group on an empty stomach in the early morning of the next day after admission and 6 months after treatment, centrifuged at 3000r/min for 10min. Serum cystatin C was detected by immunoturbidimetry, the expression of serum TL1A was detected by ELISA, and the detection was carried out in strict accordance with the operation instructions of the kit. In addition, 24-hour urine protein quantitation, plasma albumin, serum creatinine (Scr), serum urea nitrogen (BUN) and serum complement C3 were detected before and after treatment.

1.4 Statistical methods

In this study, SPSS20.0 was used for statistical analysis of experimental data. Chi-square test was used for the counting data, and mean±standard deviation was used for the measurement data. T test was used for comparison between the two groups, paired T test was used for comparison before and after treatment, and GraphPad Prism 6 software was used for drawing the experimental pictures. A p value

lower than 0.05 was considered to indicate a statistical difference.

2. RESULTS

2.1 General information

There was no significant difference in gender, age and BMI between the two groups (P>0.05), and there was no significant difference in gender, age, 24-hour urine protein quantitation, plasma albumin, complement C3, Scr, BUP, and SLEDAI score before treatment (P>0.05), which was comparable. More details were shown in Table I.

Table I General data table

Factor	Research group n=64	Control group n=64	t/X^2	P
Gender			0.125	0.724
Male	34 (53.13)	32 (50.00)		
Female	30 (46.87)	32 (50.00)		
Age (years)	36.26±7.19	36.53±7.15	0.211	0.833
BMI (kg/m^2)	21.73±2.06	21.68±2.11	0.135	0.893
History of smoking			0.027	0.869
Yes	38 (59.38)	39 (60.94)		
No	36 (56.25)	35 (54.69)		
Systolic pressure (mmHg)	127.25±11.34	125.14±9.25	1.153	0.251
Diastolic pressure (mmHg)	81.75±8.02	79.59±7.05	1.618	0.108
White blood cells ($x10^9/L$)	6.11±1.32	5.96±1.25	0.660	0.510
Platelets ($x10^9/L$)	239.45±62.18	243.65±59.37	0.391	0.697
SLEDAI (score)	19.52±0.11	-	-	-
Renal pathological grading			-	-
III	21 (32.81)	-		
IV	16 (25.00)	-		

III-V	17 (26.56)	-
IV-V	10 (15.63)	-

2.2 Expression of serum cystatin C and TL1A

The expression levels of serum cystatin C and TL1A in the research group before treatment were significantly higher than those in the control group (P<0.05), the expression levels of serum cystatin C and TL1A in the research group after treatment were significantly lower than those before treatment, but still higher than those in normal people (P<0.05), as shown in Figure 1.

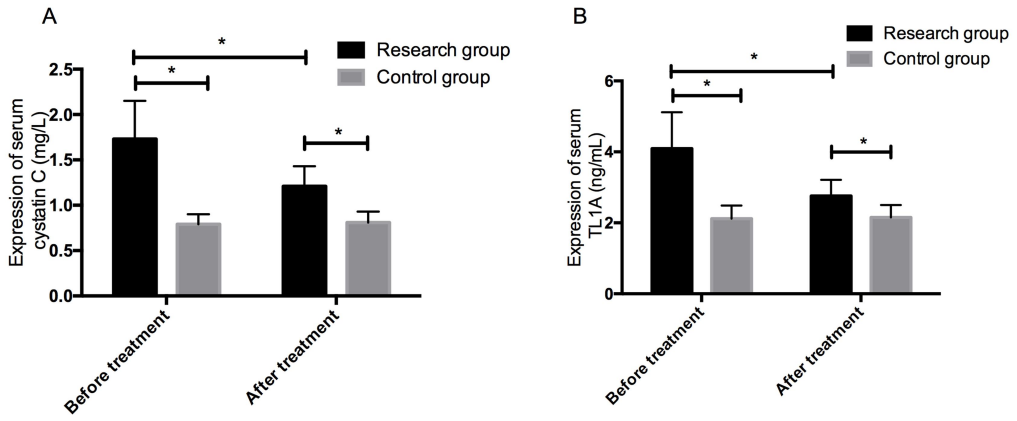

Figure 1 Expression of serum cystatin C and TL1A
Figure A: expression of serum cystatin C before and after treatment
Figure B: expression of TL1A before and after treatment
* indicated P<0.05.

2.3 Detection and evaluation of other indexes before and after treatment

After treatment, 24-hour urine protein quantitation, Scr, and BUN of the patients in the research group were significantly lower than those before treatment, and plasma albumin and C3 were significantly higher than those before treatment (P<0.05). After treatment, Scr, BUN, plasma albumin, and C3 of the patients in the research group had no significant difference with the control group (P>0.05), but 24-hour urine protein quantitation was still higher than the control group (P<0.05), as shown in Figure 2.

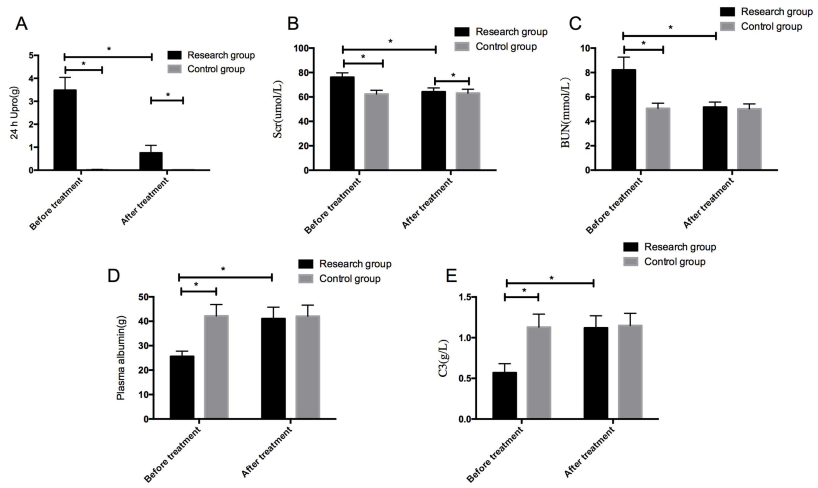

Figure 2 Detection and evaluation of other indexes before and after treatment (Figure A: 24-hour urine protein quantification before and after treatment, Figure B: Scr before and after treatment, Figure C: BUN before and after treatment, Figure D: serum albumin before and after treatment, Figure E: complement C3 before and after treatment

* indicated P<0.05.

2.4 Predictive value of serum cystatin C and TL1A for efficacy

We divided the patients into effective group and ineffective group according to their different efficacy, of which 49 patients were effective and 15 patients were ineffective. It was found that the expression levels of cystatin C and TL1A in serum of the patients in the effective group were significantly lower than those in the ineffective group before treatment, and the difference was statistically significant (P<0.05). ROC analysis showed that the predicted AUC of serum cystatin C for ineffective treatment was 0.917, and the predicted AUC of TL1A for ineffective treatment was 0.860, both of which had high predictive value, as shown in Figure 3.

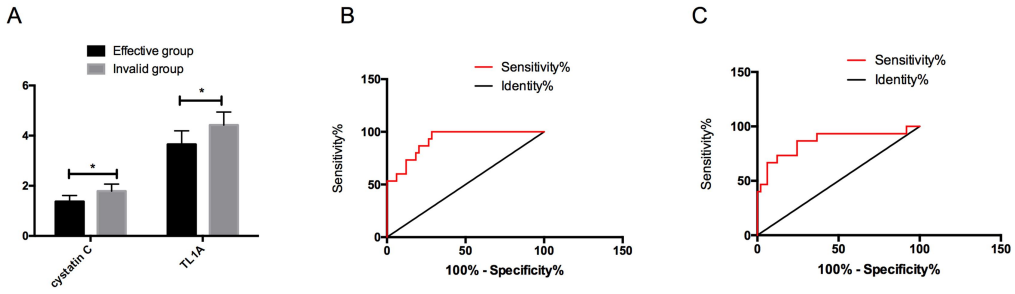

Figure 3 Predictive value of serum cystatin C and TL1A for efficacy

Figure A: expression levels of serum cystatin C and TL1A in serum of patients with different efficacy

Figure B: predictive value of serum cystatin C for efficacy
Figure C: predictive value of TL1A for efficacy
* indicated P<0.05.

2.5 Predictive value of serum cystatin C and TL1A for poor prognosis

We followed up the patients for 3 years. According to whether LN patients entered the end stage of renal disease (uremia), they were divided into good prognosis group (40 cases) and poor prognosis group (24 cases). After detecting the expression levels of cystatin C and TL1A in serum of patients in the two groups after treatment, it was found that the expression levels of cystatin C and TL1A in serum of the patients with good prognosis were significantly lower than those with poor prognosis (P<0.05). After drawing ROC curve, predicted AUC of cystatin C for LN patients with poor prognosis was 0.855, and predicted AUC of TL1A for LN patients with poor prognosis was 0.850, all of which had high predictive value, as shown in Figure 4.

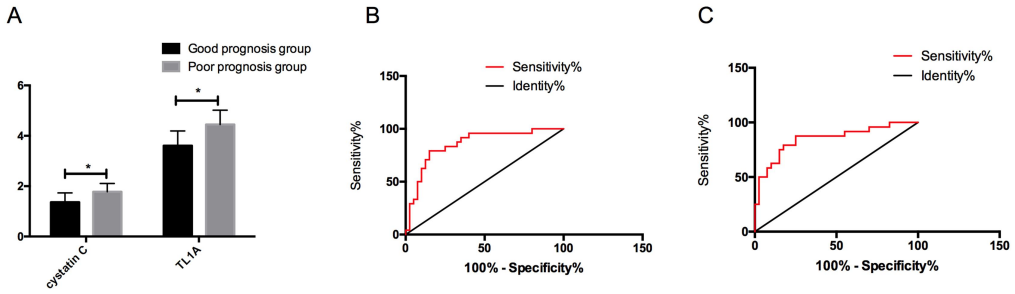

Figure 4 Predictive value of serum cystatinc C and TL1A for poor prognosis Figure A: expression of cystatin C and TL1A in serum of patients with different prognosis
Figure B: predictive value of cystatin C for poor prognosis
Figure C: predictive value of TL1A for poor prognosis
* indicated P<0.05.

2.6 Univariate analysis of poor prognosis in LN patients

According to univariate analysis of patients with good prognosis and those with poor prognosis, we found that there were no significant differences in gender, age, Scr, BUN, C3, ESR and other aspects between the two groups (P>0.05). There were significant differences in renal pathological grading, 24-hour urine protein quantification, serum cystatin C and TL1A (P<0.05). More details were shown in Table II.

Table II Univariate analysis of poor prognosis in LN patients

Factor	Good prognosis group (n=40)	Poor prognosis group n=24	t/χ^2	*P*
Gender (n,(%))			0.017	0.897
Male	21 (52.50)	13 (54.17)		
Female	19 (47.50)	11 (45.83)		
Age (years)	36.17±7.05	36.28±7.09	0.060	0.952
Renal pathological grading			13.42	0.004
III	18 (45.00)	3 (12.50)		
IV	12 (30.00)	4 (16.67)		
III-V	6 (15.00)	11 (45.83)		
IV-V	4 (10.00)	6 (25.00)		
24-hour urine protein (g)	0.42±0.18	0.89±0.15	10.74	<0.001
Scr (umol/L)	65.72±3.42	64.95±3.52	0.863	0.392
BUN (mmol/L）	5.12±0.39	5.15±0.41	0.292	0.771
Plasma albumin (g)	42.31±4.59	42.65±4.71	0.284	0.777
C3 (g/L)	1.13±0.12	1.09±0.11	1.331	0.188
Serum cystatin C (ml/l)	1.36±0.37	1.78±0.33	4.573	<0.001
TL1A (ng.ml)	3.61±0.58	4.45±0.57	5.645	<0.001

2.7 Multivariate analysis of poor prognosis in LN patients

We included renal pathological grading, 24-hour urine protein quantitation, serum cystatin C and TL1A into analysis, and listed them as dependent variables for assignment (Table III). Logistic regression model was used for multivariate analysis, and the results showed that renal pathological grading, 24-hour urine protein quantitation, serum miR-1231 and miR-192 were independent risk factors for patients with poor prognosis (Table IV).

Table III Assignment table

Factor	Assignment

Renal pathological grading	III, IV=1; III-V, IV-V=2
24-hour urinary protein quantification	Data belong to continuous variables and are analyzed with original data.
Serum cystatin C	Data belong to continuous variables and are analyzed with original data.
TL1A	Data belong to continuous variables and are analyzed with original data.

Table IV Multivariate analysis of poor prognosis in LN patients

Factor	β	S.E	Wald	OR	95%CI	P
Renal pathological grading	1.155	0.518	4.785	3.204	1.115-9.147	<0.05
24-hour urine protein quantitation	1.015	0.338	9.411	2.742	1.435-5.204	<0.05
Serum cystatin C	0.018	0.065	3.674	1.395	1.033-1.702	<0.05
TL1A	0.193	0.425	7.164	1.233	1.165-4.405	<0.05

3. DISCUSSION

LN is the basis of systemic lupus erythematosus comorbid with kidney injury. Its pathogenesis is closely related to the formation of immune complexes and various immune abnormalities in the body [12]. At present, the primary treatment for LN is drug therapy. Prednisone, as a commonly used hormone clinically, can regulate the immune state of the body. Although hormone drugs have good efficacy, they also have many side effects. Therefore, a small amount of hormone drugs are often used together with immunosuppressants in clinical practice [13,14]. Because LN patients have a long course of disease and treatment time, once the efficacy is not good, it is necessary to find a more appropriate treatment plan, so the prediction of drug efficacy and prognosis has important clinical significance for selecting treatment plan [15].

In our study, prednisone combined with leflunomide was selected to treat LN patients. Leflunomide, as a new immunosuppressive agent, can reduce the formation of B cells and antibodies in the body through the activity of light lactate

dehydrogenase. It can also inhibit the signal transmission of cells by inhibiting the activity of tyrosine kinases, thus inhibiting the generation of fibroblasts in renal interstitium and finally relieving the fibrosis process of kidney [16,17]. Previous studies [18] also found that leflunomide not only had better efficacy on LN, but also had fewer adverse reactions and higher safety when analyzing the efficacy of leflunomide in LN. However, for LN patients, there are still some differences in the efficacy of drugs. Therefore, it is necessary to predict the efficacy and prognosis of patients from the detection of other indexes, so as to timely change the therapeutic scheme and improve the prognosis. Serum cystatin C is widely present in nucleated cells and body fluids, and it is only filtered through glomerulus, so once kidney function is damaged, the expression of serum cystatin C will rise sharply, and its expression in the body is relatively stable in general. However, when kidney function changes, its change occurs earlier than Scr, so it can have higher sensitivity to changes in kidney function [19,20]. As a member of tumor superfamily, TL1A has been shown to promote the occurrence of autoimmune diseases in many studies in the past, such as rheumatoid arthritis [21]. Since the occurrence of LN is also related to autoimmune, there has been no discussion on the role of TL1A in LN in the past.

Besides, we found that the expression levels of cystatin C and TL1A in serum of LN patients were significantly higher than those of normal people, but their expression significantly decreased after treatment, which indicated that serum cystatin C and TL1A could reflect the efficacy of LN. Then we divided the patients into the effective group and the ineffective group according to the their efficacy. After comparing the serum cystatin C and TL1A of patients in the two groups before treatment, we found that the serum cystatin C and TL1A of the patients in the effective group before treatment were significantly lower than those of without treatment. After ROC analysis, we found that the serum cystatin C and TL1A had higher predictive value for the efficacy of LN. This suggested that serum cystatin C and TL1A might be used as predictors of the efficacy of prednisone combined with leflunomide in the treatment of LN patients, but no studies had confirmed them. Then

we analyzed the predictive value of serum cystatinc C and TL1A for poor prognosis of LN patients, and the results showed that serum cystatinc C and TL1A had high predictive value for their poor prognosis and were also independent risk factors for poor prognosis of them. Serum cystatin C, as a newly emerging indicator of renal function in recent years, when its expression increases, it often means that glomerular filtration rate decreases, and the decrease of glomerular filtration rate often indicates poor prognosis of patients [22]. Previous studies [23] revealed that TL1A was less expressed under normal conditions of monocytes and dendritic cells, and its expression would increase significantly after it was stimulated by inflammation or Toll-like receptor, so the increase of TL1A expression often indicates the intensification of immune response in vivo. However, there is relatively little research on TL1A in LN at present, and its specific mechanism is not clear for the time being, which needs further research.

To sum up, serum cystatin C and TL1A have high predictive value for the efficacy and prognosis of prednisone combined with leflunomide in the treatment of lupus nephritis, and the increase of serum cystatin C and TL1A are independent predictive factors for poor prognosis of LN patients, which may provide a certain reference direction for the treatment and prognosis of them. However, there are still some limitations in this study. For example, firstly, our conclusion is different from some other studies due to the small sample size. Therefore, our conclusion may need further verification. Secondly, we have not elaborated in detail on the mechanism of serum cystatin C and TL1A in LN, which also needs to be supplemented by further basic experiments.

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