Expression of inflammasomes NLRP1, NLRP3 and AIM2 in different pathologic classification of lupus nephritis

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Abstract

Objective

Lupus nephritis (LN) is an immune-complex mediated nephritis with complicated pathogenesis. The aims of the present study were to investigate whether inflammasomes are activated in the renal pathology of LN patients and analyse the association of inflammasome activation in different classes of LN renal tissues with the disease activity.

Methods

A total of 86 patients with renal biopsy-proven chronic kidney disease admitted in Xiangya Hospital from January 2015 to August 2018 were enrolled in the present study. Immunofluorescence analysis was applied to examine NLRP1, NLRP3 and AIM3 expression in renal tissues.

Results

AIM2 was mainly expressed in glomerular cells of LN class II. No obvious positive staining of AIM2 in renal tissues was found in other LN classes. NLRP1 and NLRP3 were mainly localised in tubular cells. NLRP1 was mainly expressed in tubular cells of LN class II and class IV while NLRP3 was expressed in tubular cells of LN class IV. Moreover, NLRP3 expression level was positive correlated with the activity index (AI) score in patients with LN.

Conclusion

NLRP3, NLRP1 and AIM2 activation are involved in the progress of LN. NLRP3 activation has a positive correlation with the AI score of LN.

Key words

tubulointerstitial inflammation, lupus nephritis, NLRP1, NLRP3, AIM2

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Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterised by positive tests for auto-antibodies, complement activation, and immune-complex (ICs) deposition. SLE affects multiple organs, of which one of the most important is the kidney. Patients with SLE who develop lupus nephritis (LN) have higher morbidity and mortality rates. Lupus is associated with significant organ damage resulting from activation of innate and adaptive immune signals (1-3). Kidney biopsy is an important method to enable early diagnosis and improve the prognosis of LN. Generally, the National Institutes of Health activity and chronicity indexes are used to assess the pathological injury of LN, and class III or IV LN has significant pathological manifestations and worse prognosis (4 -6).

In SLE, autoantibodies are important resources for ICs (7). Once the ICs are deposited in a certain organ, immune cells and complement system are activated. Consequently, a large amount of proinflammatory cytokines and chemokines are released, leading to damage of local cells including epithelial and endothelial cells, which promotes the recruitment of immune cells that migrate into the tissue and then amplify the inflammation, resulting in a chronic inflammatory response (8, 9). As major mediators of the pro-inflammatory response, a number of cytokines or chemokines are known to be involved in LN, such as IL-1β, IL-18, and IFN (10-12). Among them, IL-1β and IL-18 production is known to be modulated by activation of inflammasomes, the innate immune signalling complex, suggesting that inflammasomes may contribute to LN (13).

Inflammasomes are a group of multi-component signalling platforms in the cytoplasm that control inflammatory response and anti-pathogen defense against a wide range of infection and damage signals, including pathogen-associated molecular patterns and damage-associated molecular patterns (14, 15). An inflammasome usually consists of a cytosolic sensor, for instance, nucleotide-binding domain and leucine-rich-repeat-containing (NLR) protein and AIM2-like receptor (ALR) protein, an adaptor protein and apoptosis-associated speck-like protein containing a CARD (ASC). So far, there are 22 human and 34 mouse members in the NLR family (16) as well as 4 human and 14 mouse members in the ALR or the PYHIN protein family. Among them, NLRP1, NLRP3, NLRC4 (NLR family, NOD-like receptor family pyrin domain containing protein 1, 3 and 4), and AIM2 (PYHIN family, absent in melanoma 2) can activate caspase-1 (17-19), leading to cleavage of pro-IL-1β and pro-IL-18 (20, 21). Hutton *et al.* (22) have shown that the effect of the NLRP3 inflammasome on the adaptive immunity mainly through modulation of the T helper cell subsets, skewing development in favour of Th17 and Th1 cells. Recently, a number of studies have demonstrated that NLRP3, AIM2 and NLRP1 may play pivotal roles in SLE/LN and their activation might be associated with disease activity of SLE (23-28). The purposes of the present study were to investigate whether inflammasomes are involved in the renal pathology of LN patients, elucidate the type of NLR and ALR family member in different classes of LN renal tissues and determine whether their activation is associated with disease activity.

Materials and methods

Patients

This study was approved by the Ethics Committee of Xiangya Hospital. (IRB(S) no. 2018111096). The total number of 86 patients were retrospectively recruited from the patients who were admitted in Xiangya Hospital of Central South University from January 2015 to August 2018. Written informed consent was obtained from all patients. All of the patients recruited were received renal biopsy. Among them, there were 17 IgA nephropathy (IgAN), 17 minimal lesion nephritis (MLN), and 52 lupus nephritis (LN). The histologic classes of LN according to the 2018 ISN/RSP were defined as class II (n=11), class III (n=9), class IV (n=24), and class V (n=8). The following exclusion criteria were applied: No patient had coexistence of other kidney diseases, other autoimmune diseases or infec-

tions at the time of renal biopsy according to the clinical and laboratory data.

Clinical data

Clinical data including age, sex, SLE activity index (SLEDAI) and medications before renal biopsy were collected from the patients recruited. All patients were received laboratory tests of blood including routine blood test, biochemical examination, and immunoglobulin and complement assessment (Tables I-II). Renal damage scores of different types of LN, including activity index (AI), chronicity index (CI), tubulointerstitial lesions (TIL) were also assessed. (Table III).

Preparation of renal biopsy tissue

Immediately after biopsy, the obtained renal tissue was fixed in 4% acetic paraformaldehyde and either embedded in paraffin according to standard techniques or frozen and stored at -80°C. All tissues underwent direct light microscopy and direct immunofluorescence examination for diagnostic purposes and further experiments.

Morphological analysis

Renal tissues were fixed in 4% paraformaldehyde at room temperature for 24 hrs and embedded in paraffin. Paraffin sections with a thickness of 2 μm were stained with haematoxylin and eosin (H&E), periodic acid-Schiff (PAS), periodic acid-sliver methenamine, or Masson trichrome at room temperature for 30 mins to assess the extent of renal pathological changes. Renal morphologic lesions were identified on ten randomly selected, non-overlapping specimens at 200× magnification. All specimens were analysed blindly by the same investigator. The pathological score of glomerular and tubulointerstitial morphologic lesions was assessed as followings: 1+ indicating the lesions involved up to 25% of the component considered; 2+, lesions involved 25 to 50% of the component; and 3+, lesions involved 50% or more of the component. The AI was derived from the sum of scores of individual active lesions: glomerular hypercellularity, leukocyte exudation, karyorrhexis or fibrinoid necrosis, the presence of cellular crescents, the presence of hyaline deposits, and interstitial mononuclear cell infiltration. The CI was evaluated according to the sum of the following four components: glomerulosclerosis, fibrous crescents, tubular atrophy, and interstitial fibrosis. The TIL was evaluated according to the sum of the following four components: interstitial inflammatory cell infiltration, tubular atrophy, and interstitial fibrosis (29, 30).

Table I. Clinical characteristics of the patients with LN, IgAN and MLN.

	MLN	IgAN	LN
Number, n	17	17	52
Sex (F/M)	6/11‡§	13/4†	44/8†
Age (years, mean ± SD)	32.6 ± 20.6	30.1 ± 11.4	32.5 ± 14.3
Female	47.5 ± 21.4	32.4 ± 12.1	33.8 ± 14.0
Male	24.5 ± 15.7	22.8 ± 3.3	25.0 ± 15.7
SLEDAI (median, range)	-	-	12 (4-20)
WBC ($x10^9$/L)	9.0 ± 3.6§	7.4 ± 2.6	6.3 ± 3.4†
RBC ($x10^{12}$/L)	4.7 ± 1.0§	4.4 ± 0.9	3.9 ± 0.7 †
HGB (g/L)	132.3 ± 21.5§	130.9 ± 22.3§	109.0 ± 22.5†‡
PLT ($x10^9$/L)	286.9 ± 122.5‡§	212.7 ± 51.6†	204.6 ± 66.2†
LDL	5.0 ± 1.4‡§	2.7 ± 0.9†	3.5 ± 1.4†
HDL	2.3 ± 1.6‡§	1.3 ± 0.3†	1.3 ± 1.0†
ALB (g/L)	21.7 ± 9.8‡§	39.0 ± 6.5†§	28.7 ± 7.2†‡
GLB (g/L)	26.2 ± 4.7	27.4 ± 2.8	26.8 ± 6.4
BUN (mmol/L)	6.1 ± 2.8	4.2 ± 1.4	6.5 ± 4.0
CREA (μmol/L)	81.45 ± 46.80	82.6 ± 20.6	81.5 ± 46.8
mALB /UCr	3292.0 ± 3494.0‡	462.5 ± 597.3†	1652.0 ± 2416.0
UA (μmol/L)	357.7 ± 129.4	341.2 ± 78.9	354.2 ± 99.5
C4 (mg/L)	283.1 ± 93.3‡§	202.6 ± 45.9†§	107.1 ± 83.6†‡
C3 (mg/L)	1082.0 ± 188.6	861.6 ± 128.0†§	522.7 ± 233.2†‡
IgG (g/L)	6.7 ± 6.2§	11.3 ± 2.5	11.4 ± 6.5†
IgA (mg/L)	1831.0 ± 740.8‡	2912.0 ± 572.8†	2467.0 ± 1183.0
IgM (mg/L)	1453.0 ± 767.4	1207.0 ± 376.5	1082.0 ± 673.5
Anti-dsDNA(%)	-	-	65.4%
Current medications, no. (%)			
Prednisolone	7 (41)	2 (12)	50 (96)
Cyclophosphamide	0 (0)	0 (0)	0 (0)
Mycophenolate mofetil	0 (0)	0 (0)	4 (8)
Azathioprine	0 (0)	0 (0)	1 (2)
Methotrexate	0 (0)	0 (0)	0 (0)
Tacrolimus	0 (0)	0 (0)	3 (6)
Cyclosporine	0 (0)	0 (0)	1 (2)
Hydroxychloroquine	0 (0)	0 (0)	37 (71)

Values are expressed as number (percentage) or mean ± standard deviation.
†Compared with MLN group, $p<0.05$; ‡Compared with IgAN group, $p<0.05$; §Compared with LN group, $p<0.05$.
SLEDAI: disease activity index of SLE; WBC: white blood cell; RBC: red blood cell; HGB: haemoglobin; PLT: platelet; LDL: low-density lipoprotein; HDL: high-density lipoprotein; ALB: albumin; GLB: globulin; BUN: blood urea nitrogen; CREA: creatinine; mALB/UCr: MicroAlbumin/Urine creatinine; UA: uric acid; C3: Complement 3; C4: Complement 4; IgG: immunoglobulin G.

Immunofluorescence analysis

Frozen kidney sections were fixed using 4% paraformaldehyde for 30 mins. After incubation with goat serum albumin for 1 h, tissue sections were incubated with primary antibody solution at 4°C overnight, followed by incubation with secondary antibody at room temperature for 1 h. Nuclei were stained with 4'-6-diamidino-2-phenylindole. The following primary antibodies were used: anti-NLRP3 (sc-66846 at a 1:200 dilution, rabbit polyclonal, Santa Cruz Biotechnology, USA), anti-AIM2 (ab-93015 at a 1:200 dilution, rabbit polyclonal, Abcam, USA), anti-NLRP1 (sc-166368 at a 1:100 dilution, mouse monoclonal, Santa Cruz Biotechnology, USA). Image analysis was performed using a confocal laser scanning microscope (Leica TCS SP8 X&MP) and analysed using Image-Pro Plus 6.0 software (31).

Statistical analysis

Statistical analysis of the experimental data was performed using SPSS 22.0 software. The data were presented as

mean ± SD. Measurement data were analysed with the one-way analysis of variance (one-way ANOVA). The Mann-Whitney U-test was used to compare continuous variables, and chi-square or Fisher exact test was used for categorical variables. The correlations between two variables were assessed using Spearman correlation analysis. A *p*-value <0.05 indicated that the difference was statistically significant.

Results

Clinical characteristics

Eighty-six patients enrolled in this study, with 17 in the MLN group, 17 in the IgAN group and 52 in the LN group (Table I). The female to male ratio was 6/11 in the MLN group, 13/4 in the IgAN group, and 44/8 in the LN group. No statistically significant difference in age was found among the three subgroups. White blood cell, red blood cell, and platelet counts as well as haemoglobin, C3, and C4 levels were lower in the LN group, while the IgG level was higher in the LN group. As shown in Table II, there were 11 patients in the class II group, 9 in the class III group, 24 in the class IV group, and 8 in the class V group. No statistically significant differences in age, sex and SLEDAI score were found among the four subgroups. The LDL level was 2.6±0.9 mmol/L in the class II group and 4.2±1.6 mmol/L in the class IV group; the difference was statistically significant ($p<0.05$). UA was 396.2±104.5 umol/L in the class IV group and 279.7±63.0 umol/L in the class V group, while IgA was 2130.0±955.9 mg/dL in the LN class group and 3501.0±1650.0 mg/dL in the class V group. A significant difference was observed between the class IV group and class V group in UA and IgA ($p<0.05$).

Histological study

PAS, H&E, and Masson staining revealed diffuse mesangial and endothelial cell expansion, focal glomerulosclerosis, tubular atrophy, inflammatory cell infiltration and fibrosis in LN patients. However, no obvious focal glomerulosclerosis, abnormal tubular morphology and fibrosis were found in IgAN and MLN groups (Fig. 1).

Table II. Clinical characteristics of the patients with different type of LN.

	LN II	LN III	LN IV	LN V
Number, n	11	9	24	8
Sex (F/M)	7/4	9/0	21/3	7/1
Age (years, mean ± SD)	27.6 ± 19.3	36.4 ± 16.5	32.8 ± 12.2	33.8 ± 10.1
SLEDAI (median, range)	12 (4-18)	9 (5-18)	14 (4-20)	10 (4-16)
WBC ($x10^9$/L)	7.0 ± 4.2	5.6 ± 2.2	6.3 ± 3.8	6.4 ± 2.0
RBC ($x10^{12}$/L)	4.1 ± 1.0	3.7 ± 0.7	3.8 ± 0.7	4.1 ± 0.2
HGB (g/L)	108.7 ± 30.1	107.1 ± 21.1	105.2 ± 21.6	123.1 ± 9.8
PLT ($x10^9$/L)	199.3 ± 91.9	218.7 ± 73.1	210.9 ± 56.3	177.5 ± 44.9
LDL (mmol/L)	2.6 ± 0.9[§]	3.4 ± 1.0	4.2 ± 1.6[†]	2.7 ± 0.7
HDL (mmol/L)	1.0 ± 0.5	1.4 ± 0.7	1.2 ± 0.4	2.1 ± 2.2
ALB (g/L)	31.6 ± 9.5	30.4 ± 5.4	26.4 ± 6.9	29.8 ± 4.4
GLB (g/L)	29.7 ± 7.6	25.5 ± 6.3	25.5 ± 6.1	28.0 ± 5.5
BUN (mmol/l)	5.9 ± 2.2	7.2 ± 5.1	6.5 ± 2.1	4.1 ± 0.8
CREA (μmol/L)	64.0 ± 14.2	104.4 ± 84.4	89.1 ± 40.8	56.9 ± 5.4
mALB/UCr	884.0 ± 1604.0	1598.0 ± 2138.0	2080.0 ± 2748.0	1498.0 ± 2717.0
UA (μmol/L)	331.3 ± 89.8	336.5 ± 79.1	396.2 ± 104.5[#]	279.7 ± 63.0§
C4 (mg/L)	81.3 ± 54.1	128.9 ± 82.1	102.9 ± 103.1	131.0 ± 41.8
C3 (mg/L)	404.5 ± 241.4	560.6 ± 257.1	448.5 ± 211.0	679.6 ± 221.6
IgG (g/l)	14.5 ± 6.2	10.6 ± 7.3	10.5 ± 6.8	10.7 ± 3.9
IgA (mg/L)	2830.0 ± 941.5	2005.0 ± 987.8	2130.0 ± 955.9[#]	3501.0 ± 1650.0[§]
IgM (mg/L)	1426.0 ± 822.7	1049.0 ± 597.6	958.6 ± 616.2	1015.0 ± 661.3
Anti-dsDNA (%)	64%	56%	75%	50%
Current medications, no. (%)				
Prednisolone	11 (100)	8 (89)	24 (100)	7 (88)
Cyclophosphamide	0 (0)	0 (0)	0 (0)	0 (0)
Mycophenolate mofetil	1 (9)	1 (11)	0 (0)	2 (25)
Azathioprine	0 (0)	1 (11)	0 (0)	0 (0)
Methotrexate	0 (0)	0 (0)	0 (0)	0 (0)
Tacrolimus	0 (0)	1 (11)	2 (83)	0 (0)
Cyclosporine	1 (9)	0 (0)	0 (0)	0 (0)
Hydroxychloroquine	5 (45)	4 (44)	21 (88)	7 (88)

Values are expressed as number (percentage) or mean±standard deviation.
[†]Compared with LN II group, $p<0.05$; [‡]Compared with LN III group, $p<0.05$; [§]Compared with LN IV group, $p<0.05$. [#]Compared with LN V group, $p<0.05$.

Table III. AI, CI and TIL of different type of LN.

	LN II (n=11)	LN III (n=9)	LN IV (n=23)	LN V (n=7)
AI	3.2 ± 1.4[§]	5.2 ± 2.2[§]	7.4 ± 2.6[†‡#]	2.6 ± 1.4[§]
CI	0.8 ± 1.7[§]	3.7 ± 3.5[§]	4.0 ± 2.1[†‡#]	1.4 ± 1.6[§]
TIL	1.3 ± 0.5[§]	1.9 ± 0.8[§]	2.2 ± 0.5[†‡#]	1.6±0.5[§]

Values are expressed as mean±standard deviation.
[†]Compared with LN II group, $p<0.05$; [‡]Compared with LN III group, $p<0.05$; [§]Compared with LN IV group, $p<0.05$. [#]Compared with LN V group, $p<0.05$.
AI: activity index; CI: chronic index; TIL: tubulointerstitial lesions.

Expression of AIM2 in LN

Representative AIM2 immunofluorescence staining in renal biopsy specimens in LN, IgAN, and MLN groups is shown in Figure 2. Positive staining of AIM2 was predominantly localised in the glomeruli in LN renal tissues. In contrast, very weak staining of AIM2 was found in IgAN and MLN renal tissues. Semi-quantitative fluorescence analysis showed that AIM2 expression was significantly higher in LN renal tissues than that in IgAN and MLN renal tissues (Fig. 2A). Among LN patients, renal AIM2 expression was higher in class II patients than other groups (Fig. 2B).

Expression of NLRP3 in LN

As shown in Figure 3, positive staining of NLRP3 was predominantly localised in the renal tubule fields in renal tissues of patients with LN. No obvious NLRP3 staining was found in renal tissues of patients with IgAN and MLN. Semi-quantitative fluorescence analysis

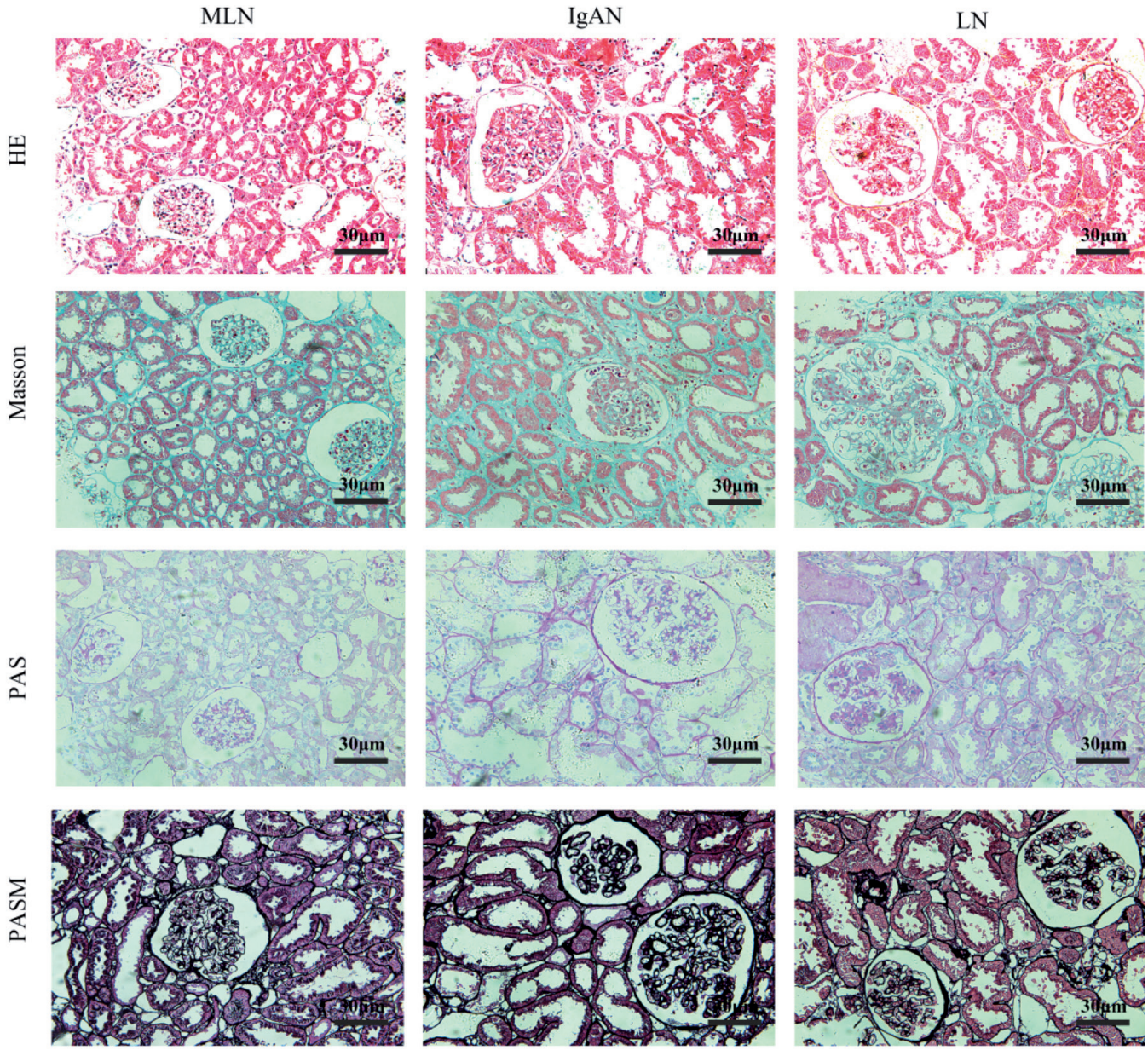


Fig. 1. General morphology analysis of renal tissues in MLN, IgAN and LN patients. Kidney sections of MLN, IgAN and LN were stained by using H&E, PAS, PASM and Masson trichrome. magnification × 200.

showed that NLRP3 level was significantly higher in LN group than that in IgAN and MLN groups (Fig. 3A). Of note, expression level of NLRP3 was higher in class IV LN patients than that in other classes of LN (Fig. 3B).

Expression of NLRP1 in LN

As shown in Figure 4, NLRP1 was predominantly expressed in the renal tubule fields in renal tissues of patients with LN. There was no positive staining of NLRP1 observed in renal tissues of patients with IgAN and MLN. Semi-quantitative analysis showed that NLRP1 level was significantly higher in LN patients than that in IgAN and MLN groups (Fig. 4A). Among LN patients, expression level of NLRP1 was higher especially in class II and class IV groups than other classes (Fig. 4B).

Correlations between clinical parameters and inflammasomes levels

As shown in Figure 5, fluorescent intensity of NLRP3 was positively correlated with AI, the activity index in LN (r=0.342, p=0.044, n=35). The correlation was stronger after excluding the data from men (r=0.413, p=0.021, n=32) (Fig. 5H). No significant correlation was found between NLRP3 level and the CI, TIL, or SLEDAI. In addition, no significant correlation was found between the expression level of NLRP1 and AIM2 and the AI, CI, TIL, SLEDAI, or other clinical parameters.

Discussion

As mentioned, a number of studies have reported that inflammasomes are involved in development and progress of SLE. For instance, expression of NLRP3 is increased in renal tissue from SLE patients and in a murine model of

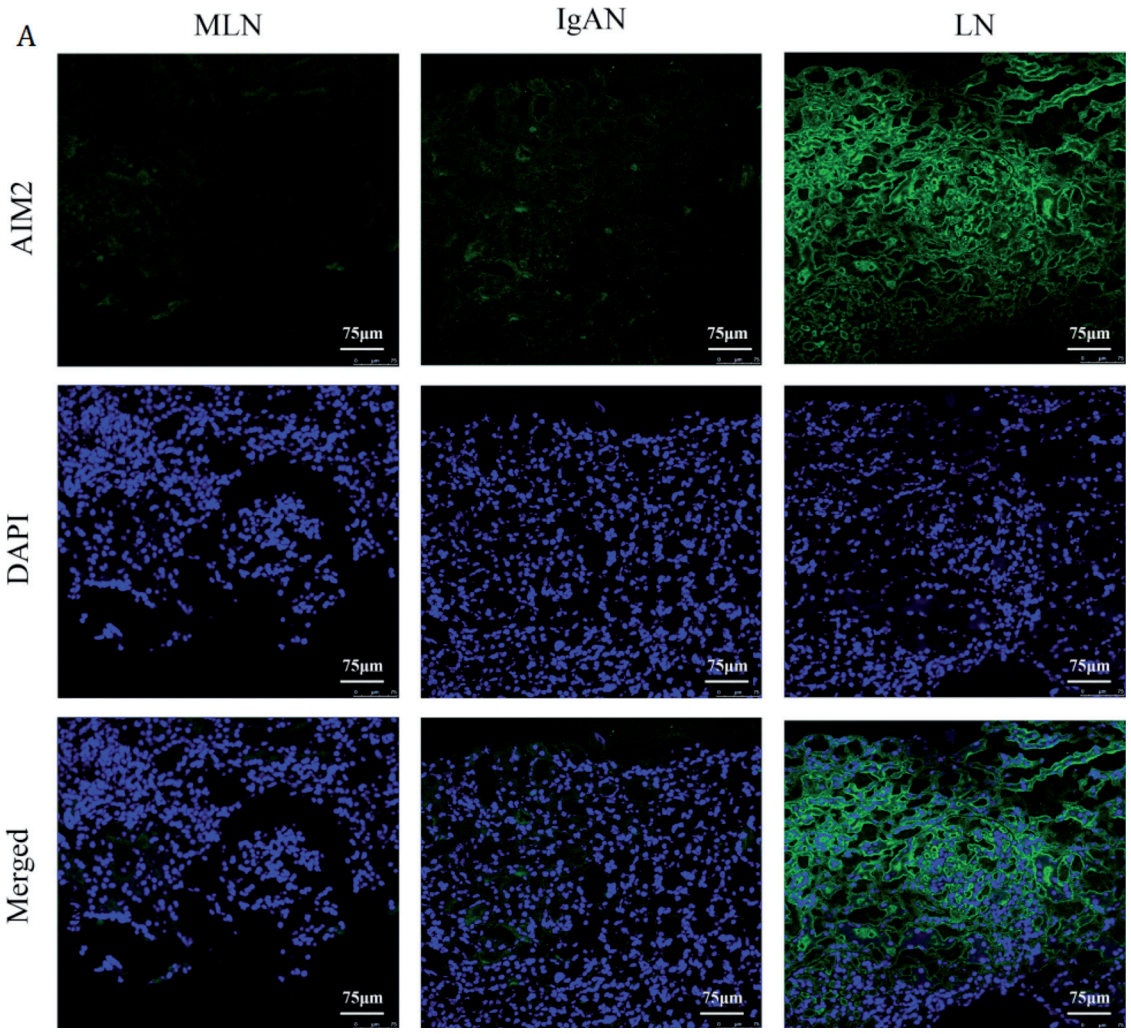
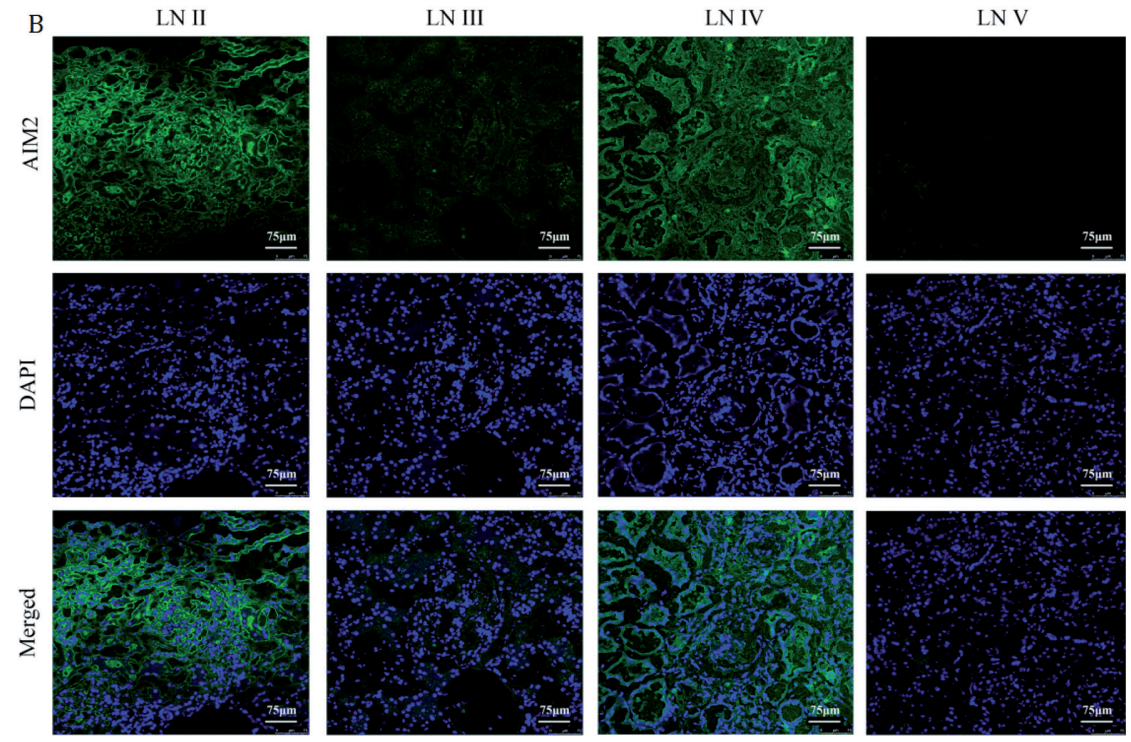


Fig. 2. Expression of AIM2 in renal tissues of MLN, IgAN and LN. Immunofluorescence analysis was performed as described in *Materials and Methods*.
A: The representative images show AIM2 expression in renal tissues of MLN, IgAN and LN.



B: The representative imges show AIM2 expression in LN renal tissues of class II, III, IV and V. Magnification×200.

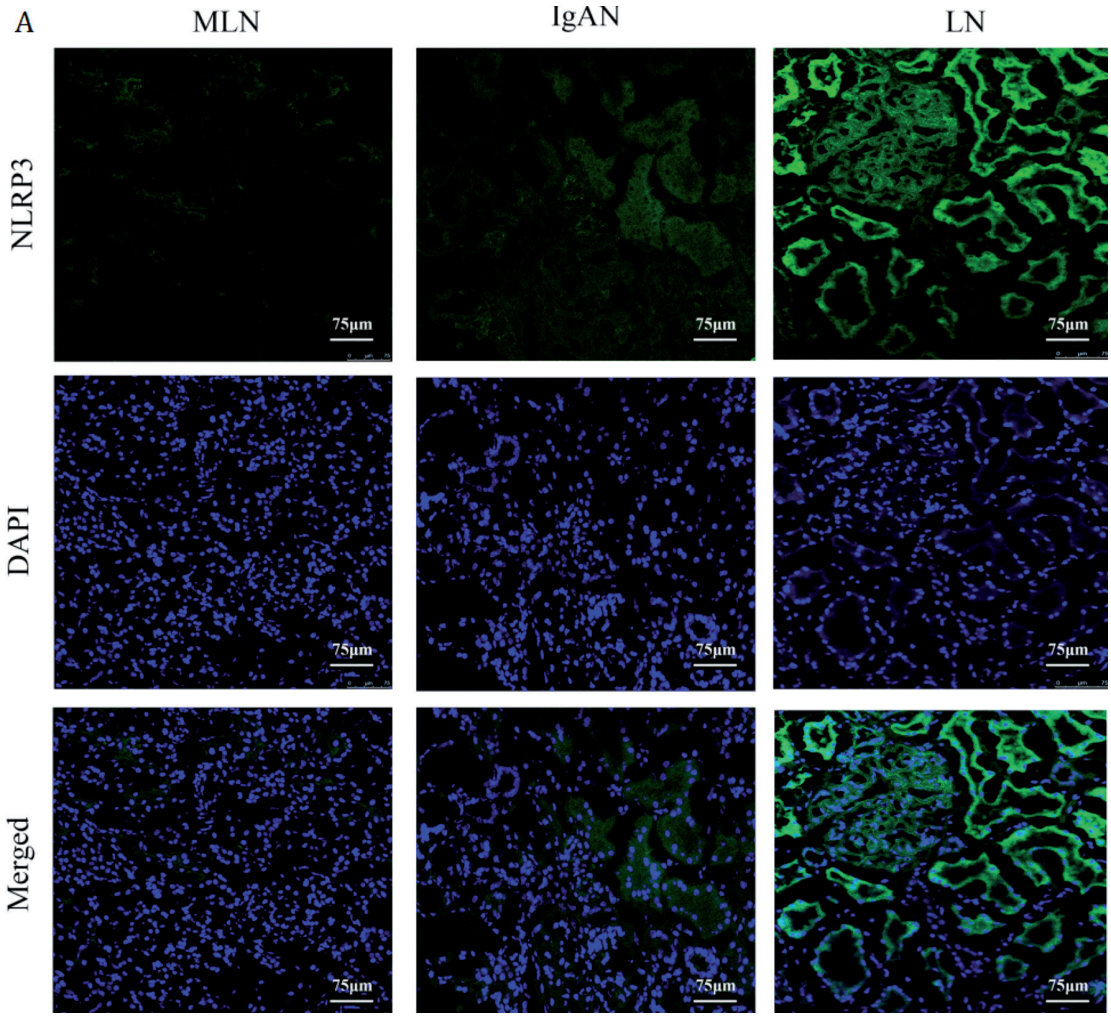
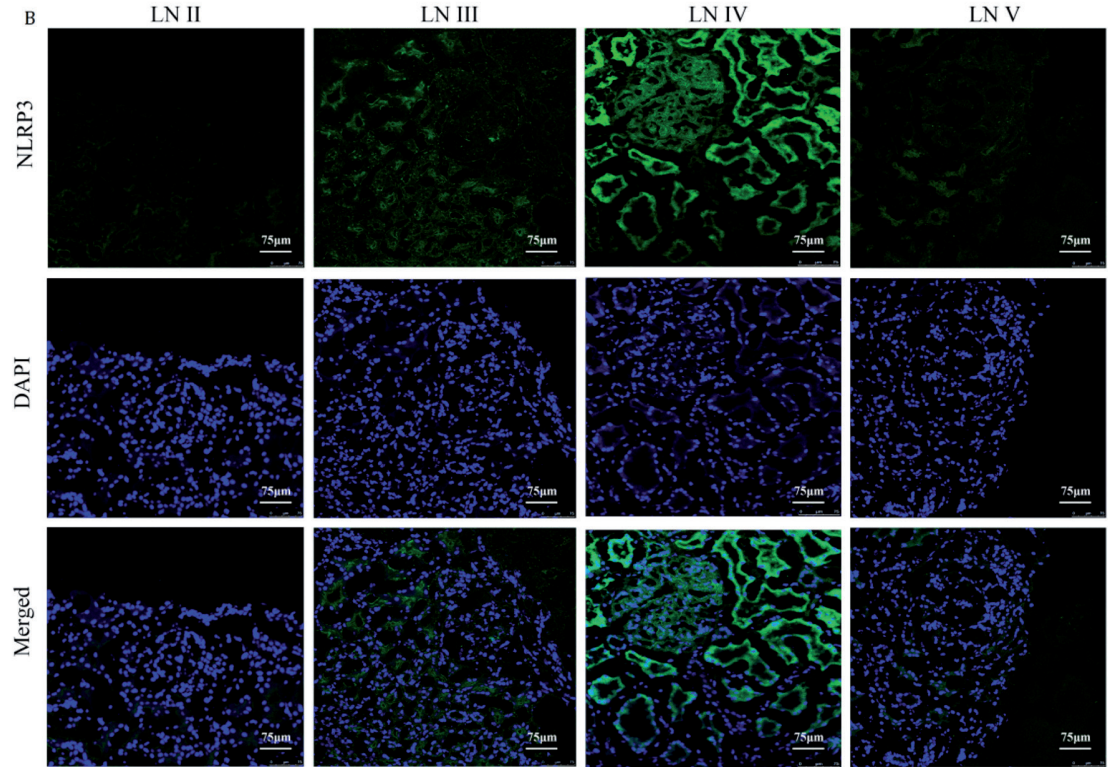


Fig. 3. Expression of NLRP3 in renal tissues of MLN, IgAN and LN. Immunofluorescence analysis was performed as described in *Materials and Methods*.
A: The representative images show NLRP3 expression in renal tissues of MLN, IgAN and LN.



B: The representative images show NLRP3 expression in LN renal tissues of class II, III, IV and V. Magnification×200.

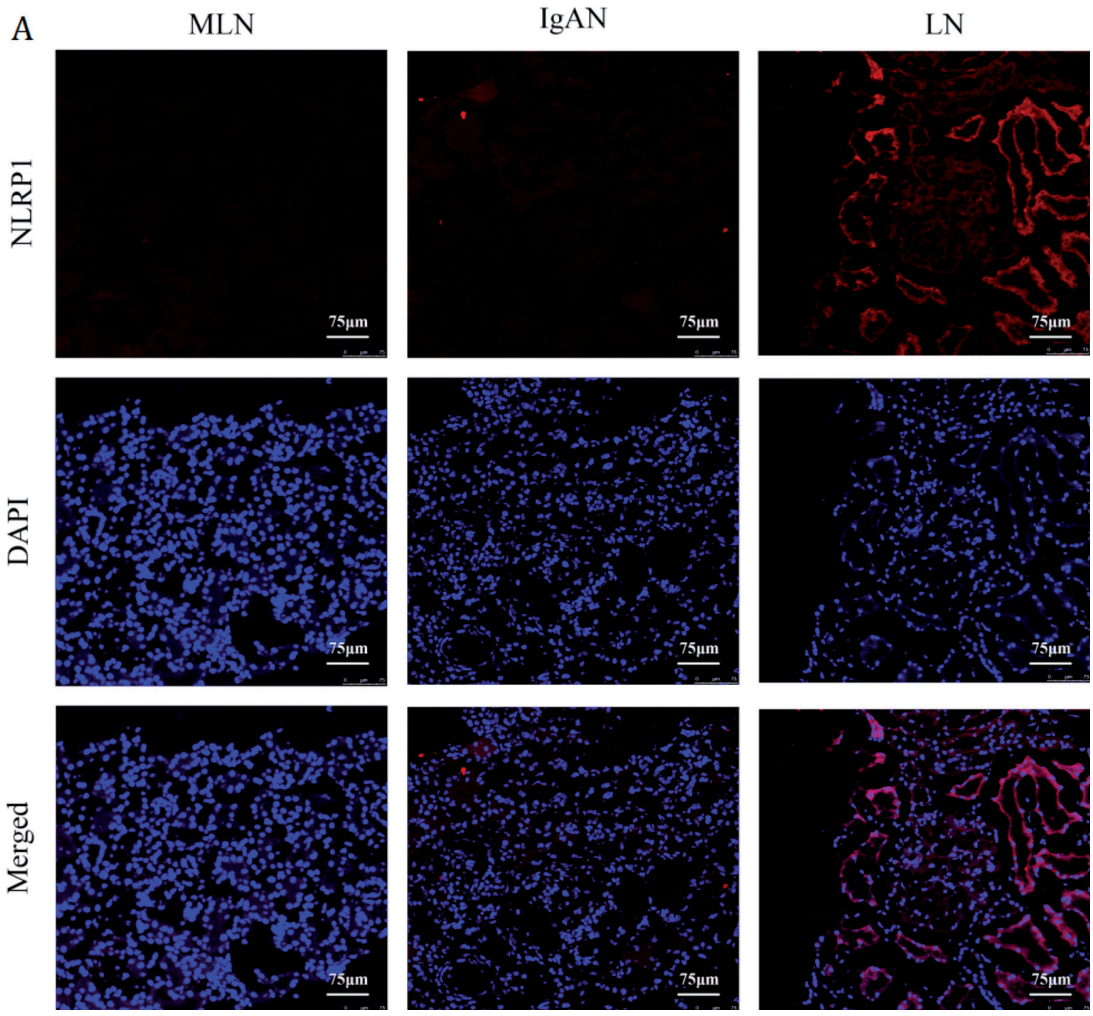
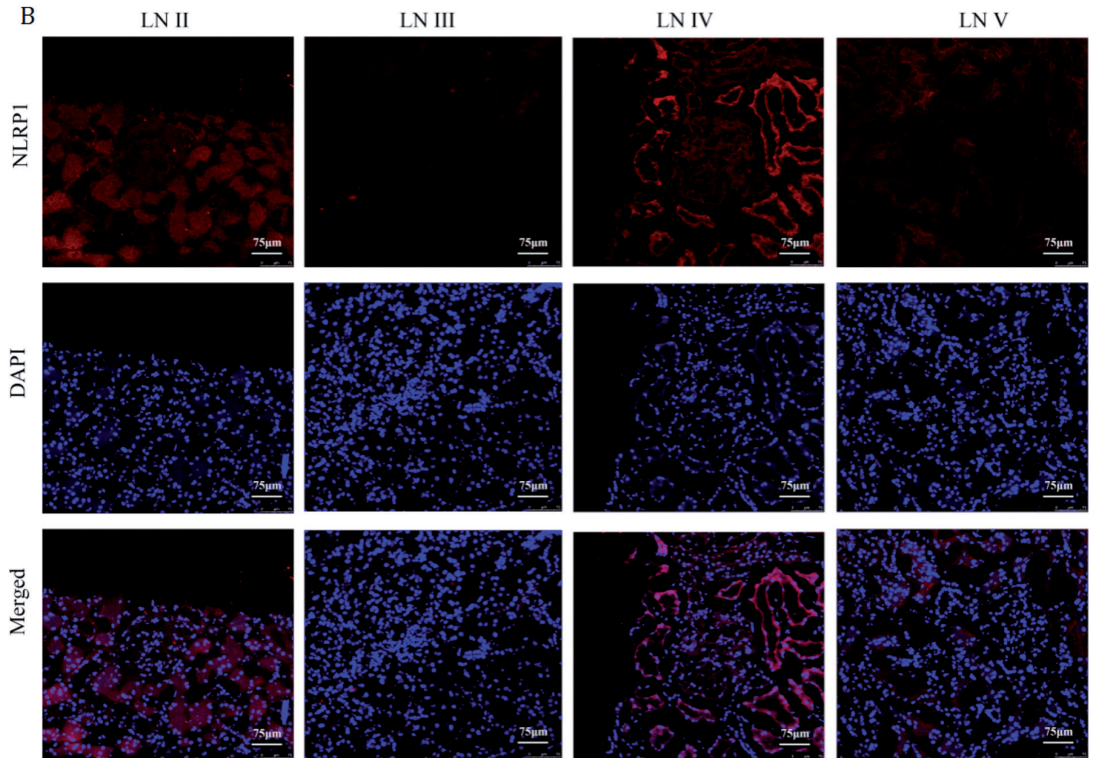


Fig. 4. NLRP1 expression in renal tissues of MLN, IgAN and LN. Immunofluorescence analysis was performed as described in *Materials and Methods*.
A: The representative images show NLRP1 expression in renal tissues of MLN, IgAN and LN.



B: The representative images show NLRP1 expression in LN renal tissues of class II, III, IV and V. Magnification×200.

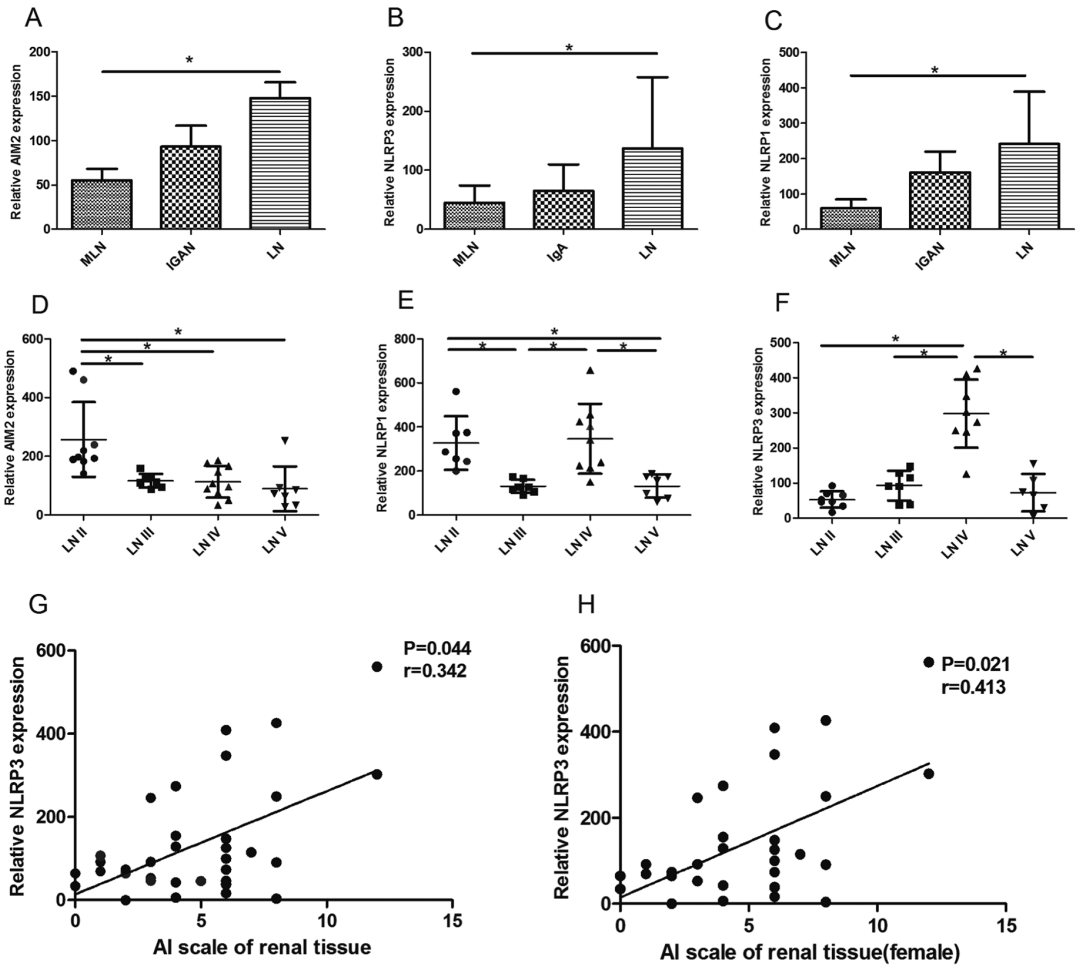


Fig. 5. Semi-quantitative analysis of AIM2, NLRP3 and NLRP1 expression in LN renal tissues.
Immunofluorescence images of AIM2, NLRP3 and NLRP1 were analysed using Image-Pro Plus 6.0 software.
A and **D**: the histogram summarised expression level of AIM2 in LN.
B and **F**: the histogram summarised expression level of NLRP3 in LN.
C and **E**: the histogram summarised expression level of NLRP1 in LN.
G: correlation between the NLRP3 level and AI.
H, the correlation analysis after excluding the data of male.
The data are expressed as mean ± SD values. *p<0.05.

lupus (32-34). Consistently, we showed that NLRP3 was upregulated in the renal tissues of LN patients. In the present study, we have also demonstrated that other inflammasome NLRP1 and AIM2 expression was increased in LN renal tissues. The localisation pattern of the above inflammasomes is different, *i.e*. AIM2 is mainly expressed in glomeruli while NLRP1 and NLRP3 mainly localised in tubular field. These data might suggest that different type of inflammasomes contributes to the inflammatory responses in different areas in LN kidney.

With regarding to the localisation of inflammasomes in human renal tissues, there are a few studies in which show NLRP3 immunostaining in renal tubular epithelial cells of diabetic nephropathy (35). However, to our knowledge, there is only one study regarding the localisation of AIM2 expression in human kidney, where it is shown that AIM2 is expressed in renal glomerular mesangial cells of hepatitis B virus-associated glomerulonephritis (37). Nevertheless, our findings that AIM2 is predominantly expressed in glomeruli while NLRP3 is mainly expressed in tubular cells are consistent with the above studies. To our knowledge, there is no study about NLRP1 and NLRC4 expression in human renal tissues. Actually, in our preliminary studies, no obvious positive staining of NLRC4 was found in renal tissues (data not shown).

As for the correlation between inflammasome activation and disease activity, Yang's group have demonstrated that expressions of NLRP3/NLRP1 inflammasomes are significantly downregulated in peripheral blood mononuclear cells (PBMCs) from patients with SLE and mRNA levels of NLRP3 and ASC are inversely correlated with the disease activity of SLE (36, 38). In the present study, we found that NLRP3 expression level in kidney is positively correlated with disease activity. Taken together, it might suggest that the NLRP3 inflammasome in circulatory immune cells and renal tissues play differential roles in the progress of SLE. For AIM2 and NLRP1 expression in LN renal tissues, no correlation of these factors with disease activity was found. However, these data should be further confirmed in the study with a large scale of patients recruited.

Of note, in the present study, we found that NLRP1, NLRP3 and AIM2 show differential activation in different class of LN, *i.e*. AIM2 shows higher in class II, NLRP1 shows higher in class II and IV and NLRP3 shows higher in class IV, which might suggest that AIM2, NLRP1 and NLRP3 are involved in different stages of LN. However, due to the limited cases, these data require to be confirmed in the study with a large scale of cases.

Previous studies on LN mainly focus on inflammation of the glomerulus. In the present study, we found that NLRP1 and

NLRP3 are mainly expressed in tubular field of LN renal tissues. However, the activation of NLRP3 inflammasomes in the tubular and interstitial cells could be mechanisms responsible for acute and chronic kidney injuries caused by ischaemia reperfusion injury, drugs, rhabdomyolysis, glucose, crystals, and unilateral nephrectomy (39-41). Recent studies have indicated that tubulointerstitial inflammation (TII) is prognostically more meaningful than glomerular inflammation (43). TII is an independent risk factor of renal outcomes. Severity of TII is associated with greater risk of end-stage renal disease in patients (42, 43). The current assessments of TII are largely qualitative, with severity scored as the fraction of the tubulointerstitial infiltrated inflammatory cells on periodic acid-Schiff-stained paraffin-embedded sections (43, 44). Although we found that NLRP3 and NLRP1 expression was increased in the tubular cells, NLRP3 and NLRP1 expression was not correlated with AI, CI, TIL, SLEDAI, or other clinical parameters in LN patients. Nevertheless, these results should be further confirmed by follow-up investigations.

Table II shows that LDL, UA, and IgA were significantly higher in class IV LN. We found that in LN patients, UA level was positively correlated with creatinine, urea nitrogen, renal pathological classification, and renal pathological injury score such as AI and CI. UA can also act as a damage-associated factor, and is released from ischaemic tissues and dying cells and is able to activate the NLRP3 inflammasome (45, 46). Thus, it may let us suggest that NLPR3 activation in LN is associated with increased UA levels.

In conclusion, NLRP1, NLRP3, and AIM2 inflammasomes are activated in renal tissues of LN. NLRP3 expression level is positively correlated with disease activity of LN. Our data would get deep insight of renal pathology of LN, thus leading to more accurate therapy for LN.

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