

Association of serum uric acid with lupus nephritis in systemic lupus erythematosus

Zaixing Yang · Yan Liang · Weihua Xi · Ye Zhu ·
Chang Li · Renqian Zhong

Received: 22 September 2009 / Accepted: 27 January 2010 / Published online: 14 February 2010
© Springer-Verlag 2010

Abstract The aim of the present study is to assess the association of elevated serum uric acid (UA) with lupus nephritis (LN) in systemic lupus erythematosus (SLE) patients. A total of 130 SLE patients were recruited, of whom 73 patients developed LN. Blood samples were obtained for determination of uric acid, complement 3 (C3), C-reactive protein (CRP) and some autoantibodies including anti-double-stranded DNA, -Smith, -SSA, -SSB, -U1RNP, SCL-70, and -Jo-1 antibodies. Correlations of UA with LN were assessed. UA was an independent risk factor for LN [odds ratio (95% CI): 1.01 (1.005–1.014); $P = 0.0000$]. The best cut-off value for UA using the ROC curve was 330 $\mu\text{mol/L}$ (sensitivity 78.1% and specificity 75.4%) and the area under the ROC curve was 0.803 ± 0.039 (95% CI: 0.727–0.878, $P = 0.000$). Spearman's correlation coefficient analysis showed negative association of UA with C3 in SLE patients with LN ($r = -0.356$, $P = 0.002$), but no association in those without LN. No correlations were found between UA and age, SLE-DAI, CRP, IgG, IgM or IgA. Furthermore, analysis of covariance demonstrated that anti-Sm ($\beta = -0.218$, $P = 0.004$) and -U1RNP ($\beta = 0.177$, $P = 0.008$) autoantibodies were independent determinants of serum UA. The

UA level is independently associated with the development of LN in SLE patients.

Keywords Lupus nephritis · Systemic lupus erythematosus · Uric acid

Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune disease of unknown etiology, characterized by chronic immune activation and multiple immunologic phenotypes [1], which predominantly affects women between the ages of 15 and 40 [2, 3]. SLE can involve various organ systems, of which kidney involvement is a major concern, affecting ~50% of patients and accounting for significant morbidity and mortality in western countries, and the 5-year survival in SLE patients with renal involvement is very low even with treatment [4]. However, it has been reported that early diagnosis and prompt treatment may dramatically modify the course of renal disease and improve the long-term survival [5]. Therefore, early detection and diagnosis of lupus nephritis (LN) appear to be of great importance.

Uric acid (UA) is a breakdown product of ingested and endogenously synthesized purines, which undergoes no further metabolism in humans and is excreted by the kidneys and the intestinal tract. Serum UA level is influenced by factors such as renal excretion function, states of high cell turnover, ingestion of food and drinks containing purines, etc. It is well known that UA crystals are the causative agents of gout. Recently, it has been demonstrated that UA is capable of activating the NLRP3 inflammasome, which plays an important role in some inflammatory responses including gout [6]. However, a number of epidemiologic

Z. Yang and Y. Liang contributed equally to this work.

Z. Yang · Y. Liang · Y. Zhu · C. Li · R. Zhong (✉)
Department of Laboratory Diagnostics, Changzheng Hospital,
Second Military Medical University, 415 Fengyang Road,
Shanghai, China
e-mail: rqzhong@yahoo.com

W. Xi
Department of Information Center, Changzheng Hospital,
Second Military Medical University, Shanghai, China

studies have reported that high UA levels in serum are associated with a wide variety of disorders, such as cardiovascular disease (CVD), hypertension, diabetes, insulin resistance, metabolic syndrome, and so on [7]. Furthermore, it has been found that although 29% of SLE patients were hyperuricemic, gout has rarely been reported in SLE patients [8]. On the other hand, some cases of coincidental SLE and gout had been reported associated with nephropathy, but the detailed association between elevated serum UA level and the development of lupus nephritis in SLE patients without gout remained unclear [9, 10]. Accordingly, this study was designed to assess the relationship between serum UA level and LN in SLE patients and the predictive value of hyperuricemia in the development of LN in the Chinese population.

Materials and methods

Patient population

Unrelated Chinese patients with SLE were recruited from the Rheumatology, Dermatology, and Nephrology Clinics of Changzheng Hospital, which is affiliated to the Second Military Medical University. The study was approved by the Research Ethics Board of Changzheng Hospital. All SLE patients recruited into the study met the 1997 revised American College of Rheumatology (ACR) SLE criteria [11]. Of all patients, those with diabetes mellitus, hypertension, and CVD were excluded from this study, because the UA level could be influenced by these diseases. After exclusion, a total of 130 SLE patients were recruited into this study of which 73 fulfilled the ACR criteria for LN [12].

Methods

All patients underwent routine laboratory assessments at their clinic visit. Blood samples were obtained for determination of serum UA, complement 3 (C3), C-reactive protein (CRP) and some autoantibodies including anti-double-stranded DNA (dsDNA), -Smith, -SSA, -SSB, -U1RNP, SCL-70, and -Jo-1 antibody. The systemic lupus erythematosus disease activity index (SLEDAI) score [13] was determined for each patient at the time of the blood draw.

Serological tests included UA measured by the enzymatic method (Hitachi Modular P800, Roche Diagnostics, Indianapolis, IN), C3 and CRP measured by nephelometry (BN, Behring, Germany), antibodies to dsDNA determined via the indirect immunofluorescence method with *Crithidia luciliae* as the substrate (EUROPIN, Germany), and antiextractable nuclear antigen (ENA) antibodies determined by immunoenzyme dot assay (EUROPIN, Germany).

Statistical analysis

All the statistical calculations were performed using SPSS 10.0 statistical software. Normally distributed variables were summarized using the mean \pm SD, and non-normally distributed variables were shown by the median with interquartile range (IQR). Selected characteristics of all participants at baseline were compared using the chi-square test for categorical variables, Student's *t* test for normally distributed continuous variables and Mann–Whitney *U* test for non-normally distributed continuous variables. Spearman's correlation coefficient was used for assessment of the correlation between two non-normally continuous variables. To estimate the effects of various factors on LN, univariate logistic regression was used to calculate odds ratios with 95% CI for UA and other risk factors. A receiver operating characteristic (ROC) curve was plotted and the area under the ROC curve was calculated to assess the diagnostic strength of uric acid for LN. Analysis of covariance (ANCOVA) was used for comparisons of serum UA levels after logarithmic transformation between the groups, with age as covariate. *P* values less than 0.05 were considered significant.

Results

Clinical and laboratory characteristics of SLE patients with or without LN are shown in Table 1. The SLE patients with LN were significantly younger than those without LN ($P = 0.003$). The SLEDAI scores and the levels of serum UA were significantly higher in SLE patients with LN than in those without ($P = 0.000$). In contrast, the levels of serum C3 were significantly lower in SLE patients with LN than in those without ($P = 0.000$). The positive rate of anti-dsDNA antibody was significantly higher in SLE patients with LN than in those without ($P = 0.04$). However, there were no differences in other factors in Table 1 between patients with or without LN.

Considering the effects of LN on the associations between UA and other factors, the patients were divided into LN group and non-LN group. Negative association between UA and C3 was only found in LN group ($r = -0.356$, $P = 0.002$), but no association was found in non-LN group. In addition, no correlations were found between UA and age, SLEDAI, CRP, IgG, IgM or IgA (see Table 2). In univariate logistic regression analyses, serum UA and intake of cyclophosphamide (odds ratio = 5.09) were independent risk factors for LN in SLE. An increment of 1 $\mu\text{mol/L}$ in serum UA concentration was associated with a 1.01 increase in the odds for risk of LN (see Table 3).

The cut-off value was calculated for serum UA associated with increase in the development of LN. The best

Table 1 Baseline characteristics of SLE patients according to whether or not LN occurred

	SLE with LN (<i>n</i> = 73)	SLE without LN (<i>n</i> = 57)	<i>P</i> value
Female/male	61/12	53/4	NS
Mean (SD) age in years	32 (14)	40 (14)	0.003
Currently taking prednisone (%)	58 (79.5)	38 (66.7)	NS
Currently taking cyclophosphamide (%)	15(20.5)	5 (0.9)	NS
Anti-dsDNA antibody (%)	40(54.8)	21(36.8)	0.04
Anti-SSA antibody (%)	39(53.4)	33(57.9)	NS
Anti-SSB antibody (%)	10(13.7)	8(14.0)	NS
Anti-U1RNP antibody (%)	23(31.5)	11(19.3)	NS
Anti-Sm antibody (%)	17(23.3)	8(14.0)	NS
SLEDAI [Mean (SD)]	18 (6)	9 (5)	0.000
C3 [Median (interquartile range, IQR)] g/L	0.39 (0.26–0.54)	0.76 (0.51–0.90)	0.000
IgG [Median (IQR)] g/L	13.4 (8.9–19.8)	14.3 (10.2–18.0)	NS
IgM (Median [IQR]) g/L	0.46 (0.19–0.61)	1.01 (0.62–1.53)	NS
IgA [Median (IQR)] g/L	2.35 (1.73–3.00)	2.10 (1.54–2.81)	NS
Uric acid [Median (IQR)] μ mol/L	416 (338–561)	279 (217–329)	0.000
CRP [Median (IQR)] mg/L	3.19 (3.08–7.14)	3.20 (3.08–12.56)	NS

Table 2 Correlations between UA and other variables in LN or non-LN groups

	SLE with LN		SLE without LN	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
SLEDAI	0.037	0.757	0.247	0.064
C3	−0.356	0.002	−0.127	0.347
IgG	0.094	0.43	0.062	0.645
IgM	−0.068	0.569	−0.073	0.588
IgA	0.008	0.945	−0.069	0.611
CRP	0.077	0.519	−0.002	0.988

Table 3 Univariate analysis of risk factors for LN

Risk factors	Odds ratio (95%)	<i>P</i> value
Age per year	0.97 (0.94–1.01)	0.102
Male sex	2.08 (0.48–9.00)	0.326
Currently taking prednisone (Yes = 1, No = 0)	0.83 (0.28–2.44)	0.729
Currently taking cyclophosphamide (Yes = 1, No = 0)	5.09 (1.24–20.85)	0.024
Anti-dsDNA antibody	1.51 (0.55–4.16)	0.425
Anti-SSA antibody	1.03 (0.37–2.88)	0.951
Anti-SSB antibody	0.78 (0.19–3.12)	0.725
Anti-U1RNP antibody	0.63 (0.09–4.33)	0.642
Anti-Sm antibody	3.49 (0.42–29.00)	0.246
C3 per g/L	0.69 (0.18–2.59)	0.578
CRP per mg/L	1.01 (0.99–1.02)	0.489
UA per μ mol/L	1.01 (1.005–1.014)	0.000

cut-off value for UA using the ROC curve was 330 μ mol/L (sensitivity 78.1% and specificity 75.4%) and the area under the ROC curve was 0.803 ± 0.039 with 95% CI of 0.727–0.878 and $P = 0.000$ (Fig. 1). Finally, ANCOVA with age as covariate demonstrated that anti-Sm ($\beta = -0.218$, $P = 0.004$) and -U1RNP ($\beta = 0.177$, $P = 0.008$) autoantibodies were independent determinants of serum UA. There were significantly higher UA levels in SLE patients with positive anti-U1RNP antibodies (2.61 ± 0.05) than in those with negative ones (2.44 ± 0.05) ($P = 0.008$). In contrast, there were significantly lower UA levels in SLE patients with positive anti-Sm antibodies (2.42 ± 0.06) than in those with negative ones (2.63 ± 0.05) ($P = 0.004$) (Fig. 2).

Discussion

It has been demonstrated, in animal model studies, that induced hyperuricemia shows a close association with renal disease [14, 15]. In humans, it has been reported that hyperuricemia is associated with initiation and progression of renal disease, including diabetic nephropathy [16, 17]. However, so far, no studies have reported the association of elevated serum UA with development of LN in SLE. In our present study of 130 SLE patients, it was demonstrated that serum UA showed a close association with development of LN in SLE. To our knowledge, this is the first description for correlation of UA with LN.

It has been reported that anti-dsDNA antibodies play an important role in the pathogenesis of LN in SLE [18]. Some studies in animal models demonstrated that extracellular

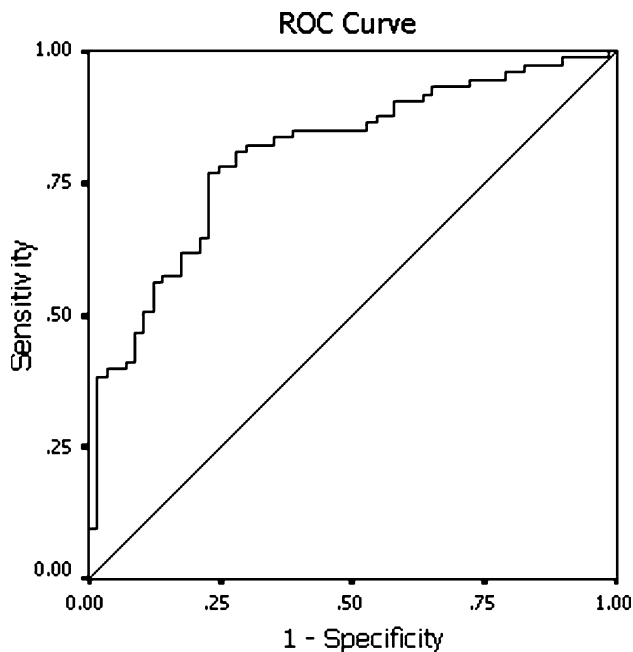


Fig. 1 ROC curve for concentrations of UA and LN

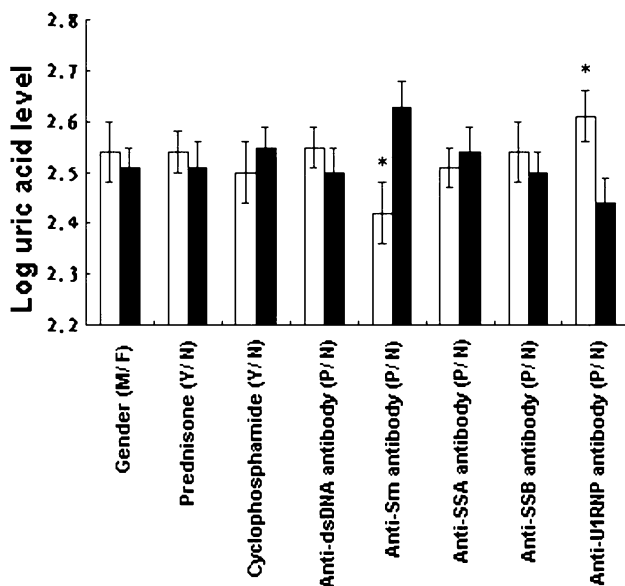


Fig. 2 Log UA levels in various SLE patients. *M* male, *F* female; *Y* yes, *N* no; *P* positive, *N* negative; White bar for male, yes or positive; Black bar for female, no or negative; Asterisk for $P < 0.01$ versus SLE patients with positive autoantibodies

dsDNA occurs mainly in the form of nucleosomes and pathogenic anti-dsDNA in SLE patients could form complexes by binding to these nucleosomes, which settle in the renal glomerular basement membrane to activate complement, initiating LN [19, 20]. In the current study, although we found there were significant differences in age, positive rate of anti-dsDNA antibody and C3 between SLE patients

with and without LN, on the other hand, after univariate logistic regression analyses, the above factors were not independent risk factors of LN. This suggests that a complex interaction between various known or unknown factors underlies their roles in development of LN. Interestingly and noticeably, among these factors, serum UA actually is an independent risk factor for development of LN in SLE, as well as its significant difference between SLE patients with and without LN. These results support the idea that UA may also be involved in the pathogenesis of nephropathy in SLE. Furthermore, intake of cyclophosphamide could be associated with the development of LN in SLE by univariate logistic regression analyses. This result seems to be inconsistent with previous reports, which reported that cyclophosphamide can be used for treatment of LN [21, 22]. However, according to the history of the patients, since most SLE patients did not take cyclophosphamide until LN occurred in China, we speculated that taking cyclophosphamide was just a therapeutic method after development of LN in SLE, but actually not a risk factor for development of LN.

In our study, serum UA showed a close association with C3 in SLE patients with LN, but not in those without LN. However, serum UA showed no associations with IgG, IgM, IgA, or CRP in both groups. The explanation might be that C3 can be activated through classical and alternative pathways by elevated UA in LN, as described in previous studies, which demonstrated that monosodium urate could induce activation of not only classical but also alternative pathway of complement in vitro [23, 24]. The deposition of complement activation products, in turn, contributes to renal tissue injury and development of LN [25]. The classical complement activation process does not require immunoglobulin, but is amplified by both CRP and IgG [26, 27]. Therefore, there might be no direct relationships between UA and CRP and Ig's, as described in the present study. In addition, there were no associations between UA and SLEDAI in both the groups, which indicated that increased serum UA had no association with SLE activity. After univariate logistic regression analyses, serum UA remained an independent risk factor of LN in patients with SLE. The size of this effect was substantial: an increase of 1 $\mu\text{mol/L}$ of serum UA led to a 1.01-fold increase in the risk of LN. Furthermore, the ROC curve of serum UA and LN from our study showed that the cut-off value for the prediction of LN was 330 $\mu\text{mol/L}$ in SLE patients, which was lower than the cut-off points used for definition of hyperuricemia (416 $\mu\text{mol/L}$ in western countries and 420 $\mu\text{mol/L}$ in China). This suggested that hyperuricemia may be defined as $\geq 330 \mu\text{mol/L}$ in Chinese population in the context of SLE, different from that in Chinese people without SLE, if it is used as a useful predictor for development of LN. Accordingly, in China, if LN is

estimated in SLE patients by hyperuricemia, the hyperuricemia might not be defined as that in general population, which should merit more attention.

In ANCONA analyses, unexpectedly, we found that serum UA was associated with the presence of serum anti-Smith and -U1RNP antibodies, but not with sex, medication (prednisone or cyclophosphamide), anti-dsDNA, -SSA, -SSB antibodies in SLE patients. Furthermore, surprisingly, SLE patients with positive anti-U1RNP antibodies showed significantly higher serum concentration of UA than the ones with negative anti-U1RNP antibodies, while SLE patients with positive anti-Smith antibodies showed significantly lower serum concentration of UA than the ones with negative anti-Smith antibodies. These novel findings provide a possible link between UA and various snRNPs, which are target antigens recognized by anti-Sm and -U1RNP autoantibodies, although the detailed mechanisms remain to be explored in future.

In conclusion, serum UA concentration can be measured easily in the clinical laboratory and applied in medical practice, and elevated serum UA level would be useful as a provisional new risk factor of LN in SLE patients. Since early diagnosis and treatment are of great importance for improvement of clinical outcome of LN [28], we propose that measurement of serum UA concentration should be used as a routine test to predict early development of LN for SLE patients. We hope that these results can provide a useful clue for further studies, although it remains to be clarified whether these findings are due to a pathogenetic role of serum UA or if this is just due to kidney damage caused by LN.

Acknowledgments This study was supported by National High-tech R & D Program (863 Program) and Funds for Excellent Department Leader, Science and Technology Commission of Shanghai Municipality.

References

1. Fairhurst AM, Wandstrat AE, Wakeland EK (2006) Systemic lupus erythematosus: multiple immunologic phenotypes in a complex genetic disease. *Adv Immunol* 92:1–69
2. Vyse TJ, Kotzin BL (1998) Genetic susceptibility to systemic lupus erythematosus. *Ann Rev Immunol* 16:261–292
3. Tsokos GC, Kammer GM (2000) Molecular aberrations in human systemic lupus erythematosus. *Mol Med Today* 6:418–424
4. Korbet SM, Lewis EJ, Schwartz MM, Reichlin M, Evans J, Rohde RD, for the Lupus Nephritis Collaborative Study Group (2000) Factors predictive of outcome in severe lupus nephritis. *Am J Kidney Dis* 35:904–914
5. Esdaile JM, Joseph L, Mackenzie T, Kashgarian M, Hayslett JP (1994) The benefit of early treatment with immunosuppressive agents in lupus nephritis. *J Rheumatol* 21:2046–2051
6. Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J (2006) Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature*. 440:237–241
7. Feig DI, Kang DH, Johnson RJ (2008) Uric acid and cardiovascular risk. *N Engl J Med* 359:1811–1821
8. Froncht A, Leek JC, Robbins DJ (1987) Gout and hyperuricemia in systemic lupus erythematosus. *Br J Rheumatol* 26:303–306
9. Ho HH, Lin JL, Wu YJJ, Yu KH, Chen JY, Luo SF (2003) Gout in systemic lupus erythematosus and overlap syndrome—a hospital-based study. *Clin Rheumatol* 22:295–298
10. Pu SJ, Luo SF, Wu YJJ, Cheng HS, Ho HH (2000) The clinical features and prognosis of lupus with disease onset at age 65 and older. *Lupus* 9:96–100
11. Hochberg MC (1997) Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. *Arthritis Rheum* 40:1725
12. Renal Disease Subcommittee of the American College of Rheumatology Ad Hoc Committee on Systemic Lupus Erythematosus Response Criteria. (2006) The American College of Rheumatology response criteria for proliferative and membranous renal disease in systemic lupus erythematosus clinical trials. *Arthritis Rheum*, 54: 421–432
13. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang DH, the Committee on Prognosis Studies in SLE (1992) Derivation of the SLEDAI: a disease activity index for lupus patients. *Arthritis Rheum* 35:630–640
14. Khosla UM, Zharikov S, Finch JL, Nakagawa T, Roncal C, Mu W, Krotova K, Block ER, Prabhakar S, Johnson RJ (2005) Hyperuricemia induces endothelial dysfunction. *Kidney Int* 67:1739–1742
15. Sanchez-Lozada LG, Tapia E, Santamaria J, vila-Casadao C, Soto V, Nepomuceno T, Rodriguez-Iturbe B, Johnson RJ, Herrera-Acosta J (2005) Mild hyperuricemia induces vasoconstriction and maintains glomerular hypertension in normal and remnant kidney rats. *Kidney Int* 67:237–247
16. Rosolowsky ET, Ficociello LH, Maselli NJ, Niewczas MA, Binns AL, Roshan B, Warram JH, Krolewski AS (2008) High-normal serum uric acid is associated with impaired glomerular filtration rate in nonproteinuric patients with type 1 diabetes. *Clin J Am Soc Nephrol* 3:706–713
17. Hovind P, Rossing P, Tarnow L, Johnson RJ, Parving HH (2009) Serum uric acid as a predictor for development of diabetic nephropathy in type 1 diabetes—an inception cohort study. *Diabetes* 58:1668–1671
18. Rahman A, Isenberg DA (2008) Systemic lupus erythematosus. *N Engl J Med* 358:929–939
19. Kramers C, Hylkema MN, van Bruggen MC et al (1994) Anti-nucleosome antibodies complexed to nucleosomal antigens show anti-DNA reactivity and bind to rat glomerular basement membrane in vivo. *J Clin Invest* 94:568–577
20. van Bruggen MC, Walgreen B, Rijke TP et al (1997) Antigen specificity of anti-nuclear antibodies complexed to nucleosomes determines glomerular basement membrane binding in vivo. *Eur J Immunol* 27:1564–1569
21. Masood S, Jayne D, Karim Y (2009) Beyond immunosuppression—challenges in the clinical management of lupus nephritis. *Lupus* 18(2):106–115
22. Karim MY, Pisoni CN, Khamashta MA (2009) Update on immunotherapy for systemic lupus erythematosus—what’s hot and what’s not!. *Rheumatology (Oxford)* 48(4):332–341
23. Giclas PC, Ginsberg MH, Cooper NR (1979) Immunoglobulin G independent activation of the classical complement pathway by monosodium urate crystals. *J Clin Invest* 63:759–765
24. Terkeltaub R, Tenner AJ, Kozin F, Ginsberg MH (1983) Plasma protein binding by monosodium urate crystals: analysis by two-dimensional gel electrophoresis. *Arthritis Rheum* 26:775–783
25. Couser WG, Baker PJ, Adler S (1985) Complement and the direct mediation of immune glomerular injury: a new perspective. *Kidney Int* 28:879–890

26. Russell IJ, Papaioanlou C, McDuffie FC, MacIntyre S, Kushner I (1983) Effects of IgG and C-reactive protein on complement depletion by monosodium urate crystals. *J Rheumatol* 10:425–433
27. Fields TR, Abrabson SB, Weissmann G, Kaplan AP, Ghebrehiwet B (1983) Activation of the alternative pathway of complement by monosodium urate crystals. *Clin Immunol Immunopathol* 26:249–257
28. Feihn C, Hajjar Y, Mueller K, Waldherr R, Ho AD, Andrassy K (2003) Improved clinical outcome of lupus nephritis during the past decade: importance of early diagnosis and treatment. *Ann Rheum Dis* 62:435–439