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Original Article

Henoch-Schonlein purpura nephritis and their effects on immune function and prognosis of patients

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Abstract: Objective: This study aimed to assess the clinical efficacy of tacrolimus and cyclophosphamide in the treatment of Henoch-Schonlein purpura nephritis (HSPN) and to investigate their effects on immune function and prognosis of patients. Methods: A total of 104 patients with HSPN who were treated in our hospital from January 2014 to June 2018 were recruited. Patients treated with tacrolimus were served as group A (n=57), and those treated with cyclophosphamide as group B (n=47). The 24 h urine protein (24 h Upro), urine red blood cell (RBC) count, blood lipid indexes, liver and kidney function indexes, immune function, clinical efficacy, as well as complication rate between the two groups were compared. Results: After treatment, the 24 h Upro and urine RBC count were reduced in both groups, and the reduction in group A was more significant than that in group B (P<0.05). The cholesterol decreased in both groups after treatment (P<0.05). The albumin was remarkably elevated in both groups after treatment, and the elevation in group A was more significant than that in group B. The serum creatine (SCr), blood urea nitrogen (BUN), and immunoglobulins (IgA, IgG, IgM) decreased significantly in the two groups after treatment, and the SCr and BUN as well as IgA in group A were significantly lower than those in group B (P<0.05). The patients in group A showed significantly higher total effective rate and complication rate than those in group B (P<0.05). Conclusion: Although both tacrolimus and cyclophosphamide are effective in treating HSPN, tacrolimus is superior to cyclophosphamide, which induces more adverse reactions, however. Therefore, it is necessary to assess the patient's condition comprehensively in order to select the right treatment.

Keywords: Tacrolimus, cyclophosphamide, Henoch-Schonlein purpura nephritis, immune function, prognosis

Introduction

Henoch-Schonlein purpura nephritis (HSPN) is a circulatory disease with vascular inflammatory responses, mainly manifested as skin purpura, abdominal and joint pain, as well as decreased renal function [1, 2]. Due to the defects in autoimmune system, the patients may present with allergic reactions such as immunoglobulin (IgA) complex deposition on the vessel wall after being stimulated by bacterial or viral infection [3]. Long-term renal function involvement may lead to purpuric nephritis. And renal injury is aggravated in disease progression due to the increase of urine protein and prolonged time; purpuric nephritis may develop into chronic renal disease and eventually end-stage renal disease [4, 5]. Because of the low specificity of existing methods, symptomatic treatment and protection of renal function are still preferred initiatives [6]. The poor prognosis and high difficulty in treatment make early and effective intervention crucial for patients with HSPN.

With the advancement of medical and molecular science, the long-term efficacy and drug safety control of immunosuppressive therapies have been significantly improved in the treatment of nephritis [7]. Tacrolimus is a novel immunosuppressive agent regulating the activity of calcineurin, which inhibits T cell proliferation *in vivo* by regulating tyrosine hydroxylase synthesis and releasing interleukin-1 (IL-2), and directly acts on the immune response induced by decreased antigen. These characteristics determine that tacrolimus can be applied in reliving rejection reaction after transplantation [8, 9]. Cyclophosphamide, a cytotoxic alkylating agent, exerts a drug action of interference with

deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) by preventing cell proliferation and survival of antigen-sensitive small lymphocytes, as well as inhibiting the transformation of immunoblasts, and its potency generally lasts for a long time [10, 11].

Both tacrolimus and cyclophosphamide are effective in the treatment of HSPN, so this study compared their efficacy and effects on the immune function and prognosis of patients.

Methods and materials

General data

A total of 104 patients 59 males and 45 females, average age 4.69 ± 0.73 years, course of disease 63.45±3.83 days with HSPN admitted to our hospital from January 2014 to June 2018 were enrolled, of which 57 patients treated with tacrolimus were assigned to group A, and 47 treated with cyclophosphamide to group B.

Inclusion and exclusion criteria

Inclusion criteria: (1) Patients meeting the diagnostic criteria and clinical classification of HSPN in *Nephrology* [12]; (2) Patients with abnormal hematuria and a 24 h urine protein (Upro) of 0.5~3.5 g/24 h. Exclusion criteria: (1) Patients with systemic lupus erythematosus, B-related nephritis and other primary or secondary renal diseases; (2) Patients receiving glucocorticoids or other immunosuppressive agents before treatment; (3) Patients with acute and chronic infection; (4) Patients with poor compliance. All patients and their families agreed to participate in the experiment and sign the informed consent form. The experiment was conducted with the approval of the Medical Ethics Committee.

Experimental reagents and materials

Tacrolimus (Astellas Pharma Co., Ltd., China, approval number: H20150195); cyclophosphamide (Jiangsu Suncadia Medicine Co., Ltd., approval number: H32024654); plasma separator (Shanghai Pudong Tianben Centrifugal Machinery Co., Ltd.); full-automatic biochemica analyzer (Shenzhen Hongsen Environmental Protection Technology Co., Ltd.); automatic urine analyzer (Shanghai Hanfei Medical Devices Co., Ltd.); chemiluminescence analyzer (Beijing Beier Bioengineering Co., Ltd.).

Methods

Treatment methods: In group A, the patients were treated with tacrolimus 0.10~0.15 mg/(kd.d) orally, 2 h after meal, twice a day, for 15 days, while those in group B received shock therapy with continuous intravenous drip of 10 mg/(kg.d) cyclophosphamide, two days for one course (once a day) for seven courses, with a cumulative dose of less than 170 mg/kg. According to the blood drug concentration and adverse drug reactions *in vivo*, drug concentration adjustment and hydration as well as alkalization of urine were carried out.

Index detection methods: Fasting venous blood samples (2 mL) were extracted from all subjects in the morning and kept standing at room temperature for 30 min. After complete coagulation, the blood was centrifuged at 4,000 r/min for 5 min. Then immunoglobulins (IgA, IgG, IgM) and other related indexes were detected with a full-automatic biochemical analyzer, serum creatine (Scr) and blood urea nitrogen (BUN) levels with a chemiluminescent analyzer, and 24 h Upro and red blood cell (RBC) count with an automatic urine analyzer. All operations were carried out in strict accordance with the instruction manual.

Outcome measures: (1) Comparison of 24 h Upro and urine RBC count; (2) Comparison of blood lipid and liver and kidney function indexes; (3) Observation of immune function; (4) Comparison of clinical efficacy: cure: 24 h Upro less than 0.2 g/24 h, RBC count of urine sediment less than 3/field of vision, and normal renal function; markedly effective: urine RBC and 24 h Upro decreased by ≥50%; effective: 24 h Upro decreased by 25%~49%, and stable renal function; ineffective: failure to meet the above criteria. Total effective rate = (cure + markedly effective + effective) ×100% [13]. (5) Comparison of the total complication rate.

Statistical methods: Statistical analysis was carried out with SPSS 19.0 (Beijing NDTimes Science and Technology Co., Ltd.). The counting data were analyzed with chi-square test and the measurement data were expressed as means ± standard deviation. Comparison between the two groups was conducted with the t test, and comparison before and after treatment was conducted with the repeated measurement analysis of variance. Graphpad Prism8 was employed to plot the figures. The

Table 1. Comparison of general data

Group	Group A n=57	Group B n=47	t/X^2	P
Sex (cases)			0.018	0.894
Male	32 (56.14)	27 (57.45)		
Female	25 (43.86)	20 (42.55)		
Age (years)	4.46 years	4.71 years	0.221	0.825
Course of disease (days)	63.43±3.87	63.49±3.82	0.079	0.937
Rash (cases)			5.963	0.994
Yes	23 (40.35)	19 (40.43)		
No	34 (59.65)	28 (59.57)		
Arthralgia (cases)			0.207	0.649
Yes	13 (22.81)	9 (19.15)		
No	44 (77.19)	38 (80.85)		
Lymphadenectasis (cases)			0.292	0.589
Yes	16 (28.07)	11 (23.40)		
No	41 (71.93)	36 (76.60)		
Hepatosplenomegaly (cases)			0.878	0.349
Yes	14 (24.56)	8 (17.02)		
No	43 (75.44)	39 (82.98)		
Headache (cases)			0.150	0.699
Yes	19 (33.33)	14 (29.79)		
No	38 (66.67)	33 (70.21)		

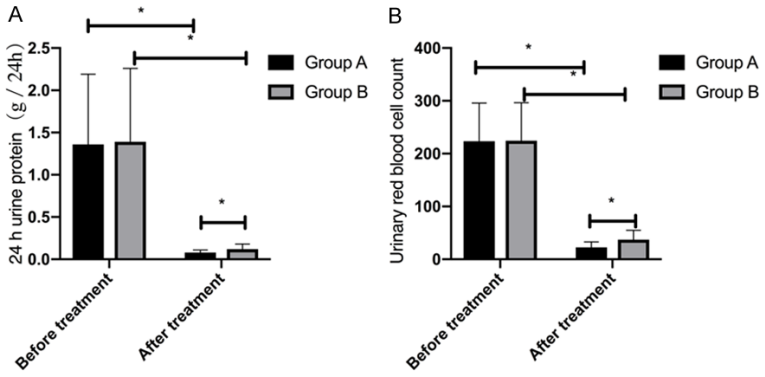

Figure 1. Comparison of 24 h Upro and urine RBC count. A. 24 h Upro decreased significantly in both groups after treatment, and group A was lower than group B. B. Urine RBC count decreased significantly in both groups after treatment, and group A was lower than group B. Note: * indicated *P*<0.05.

difference was statistically significant with *P*< 0.05.

Results

Comparison of general data

There was no difference between the two groups in terms of sex, age, course of disease, appearance of rash and arthralgia (*P*>0.05). See **Table 1** for details.

Comparison of 24 h Upro and urine RBC count

Before treatment, there was no significant difference between group A and group B in terms of 24 h Upro and urine RBC count (*P*>0.05). Whereas after treatment, both of them decreased significantly in the two groups, and the decrease in group A was more significant than group B (24 h Upro: 0.08±0.03 vs. 0.12±0.06, urine RBC count: 22.45±10.24 vs. 37.24±17.43) (*P*<0.05). See **Figure 1** for details.

Comparison of blood lipid indexes

There was no significant difference in cholesterol and triglyceride levels between the two groups before and after treatment (*P*>0.05), but they decreased significantly after treatment (*P*<0.05). See **Figure 2** for details.

Comparison of liver and kidney function indexes

There was no significant difference in liver and kidney function indexes between the two groups before treatment (*P*>0.05). Moreover, ALT showed no significant differences before and after treatment (*P*>0.05). While the group A showed significantly higher albumin than the group B (51.34±7.43 vs. 45.34±7.12), which was elevated in both groups after treatment. The group A showed significantly lower Scr and BUN than the group B (41.15±8.93 vs. 46.23± 9.12, and 4.34±0.67 vs. 4.62±0.72), and they were reduced in the both groups after treatment (*P*<0.05). See **Figure 3** for details.

Comparison of immune function indexes

There was no significant difference in IgA, IgG, IgM between the two groups before treatment as well as in IgG and IgM after treatment (*P*>

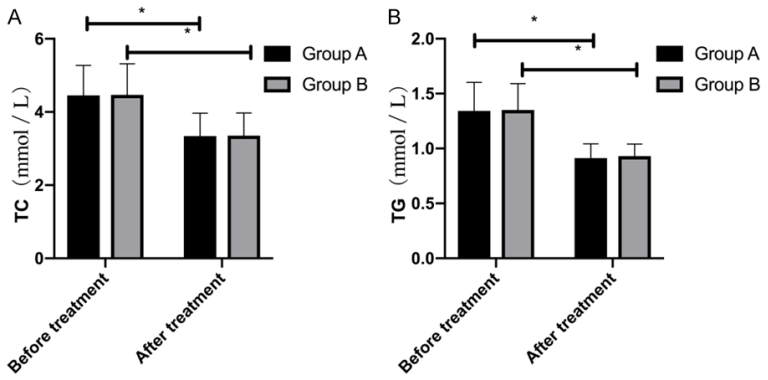

Figure 2. Comparison of blood lipid indexes. A. Cholesterol decreased significantly in the two groups after treatment. B. Triglycerides decreased significantly in the two groups after treatment. Note: * indicated $P<0.05$.

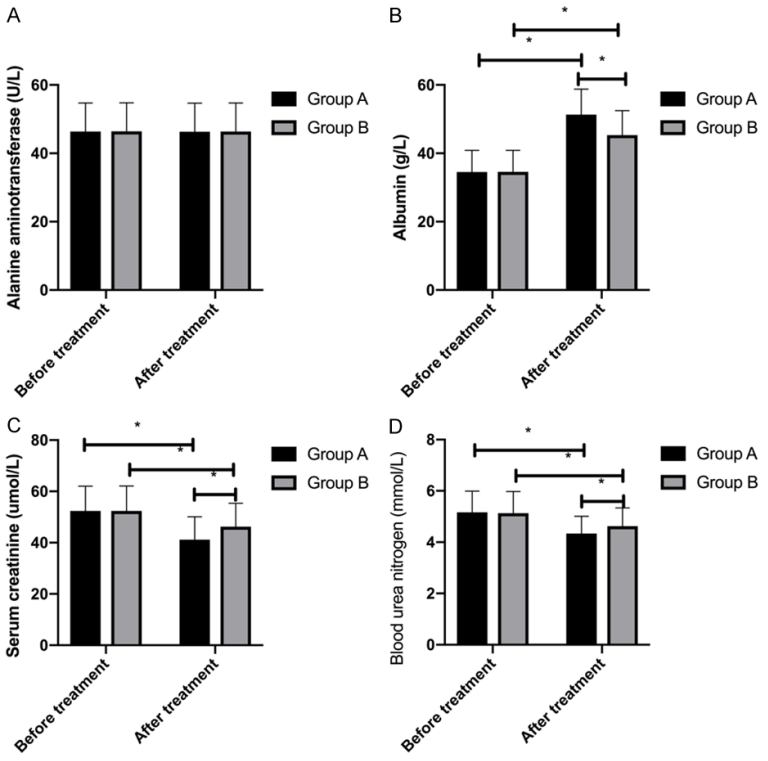

Figure 3. Comparison of liver and kidney function indexes. A. There was no significant statistical difference in ALT between the two groups before and after treatment. B. Albumin increased significantly in the two groups after treatment, and group A was significantly higher than group B. C. SCr decreased significantly in the two groups after treatment, and group A was significantly lower than group B. D. BUN decreased significantly in the two groups after treatment, and group A was significantly lower than group B. Note: * indicated $P<0.05$.

0.05). While after treatment, IgA, IgG, IgM decreased significantly in both groups, and IgA in group A was lower than that in the group B (1.34±0.42 vs. 1.72±0.47) ($P<0.05$). See **Figure 4** for details.

Comparison of therapeutic effects

After treatment, the group A showed a remarkably higher total effective rate than the group B (80.70% vs. 59.57%) ($P<0.05$). See **Table 2** for details.

Comparison of total complication rate

After treatment, the group A showed a remarkably higher total complication rate than the group B (21.05% vs. 6.38%) ($P<0.05$). See **Table 3** for details.

Discussion

Kidney damage caused by Henoch-Schonlein purpura that involves multiple organs during the progression is called HSPN, which is common in children with an annually increasing incidence in recent years [14, 15]. At present, there is no unified direction for the specific treatment of nephritis at home and abroad, and the treatment plan is mainly determined according to the individual conditions, pathological diagnosis, and clinical classification of patients [16, 17]. Patients with III-IV HSPN is found to have a worse prognosis than those in the early stages, so early and reasonable immunotherapies can effectively prevent disease from advancing to end-stage renal disease [18, 19]. However, the application of long-term and large-dose immunosuppressive agents may also induce serious complications, such as infection, leukopenia, elevated blood sugar, femoral head necrosis and myelosuppression. Moreover, a sudden interruption of the treatment may result in rapid relapse of HSPN [20]. Therefore, exploring reasonable

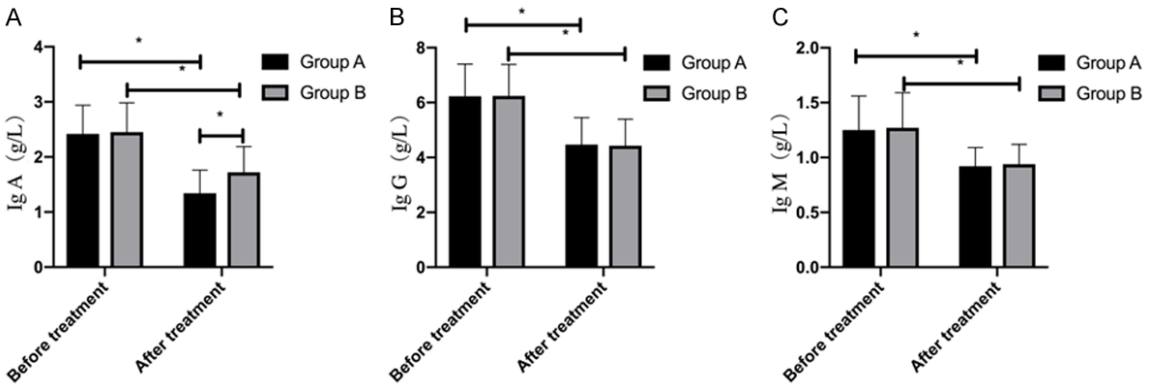

Figure 4. Comparison of immune function indexes. A. IgA decreased significantly in the two groups after treatment, and group A was significantly lower than that in group A. B. IgG decreased significantly in the two groups after treatment. C. IgM decreased significantly in the two groups after treatment. Note: * indicated $P<0.05$.

Table 2. Comparison of total effective rate (n [%])

Group	Group A n=57	Group B n=47	X^2	P
Cure	18 (31.58)	12 (25.53)	-	-
Markedly effective	22 (38.60)	11 (23.40)	-	-
Effective	6 (10.53)	5 (10.64)	-	-
Ineffective	11 (19.30)	19 (40.43)	-	-
Total effective rate	46 (80.70)	28 (59.57)	5.602	0.018

Table 3. Comparison of total complication rate (n [%])

Group	Group A n=57	Group B n=47	X^2	P
Respiratory tract infection	1 (1.75)	1 (2.13)	-	-
Shingles	2 (3.51)	0	-	-
Gastrointestinal discomfort	3 (5.26)	1 (2.13)	-	-
Liver injury	3 (5.26)	1 (2.13)	-	-
Elevated blood sugar	1 (1.75)	0	-	-
Myelosuppression	2 (3.51)	0	-	-
Total complication rate	12 (21.05)	3 (6.38)	4.491	0.034

application of immunosuppressive agents, such as tacrolimus and cyclophosphamide, is of great significance for elevating treatment of HSPN and improving immune function and long-term prognosis of the patients.

In our study, both 24 h Upro and urine RBC count decreased significantly in the two groups after treatment, and group A was significant lower than group B, which indicated that tacrolimus was more effective than cyclophosphamide in reducing 24 h Upro and urine RBC count. Besides, we found that the blood lipid indexes (cholesterol and triglyceride) decreased in the two groups after treatment. A study revealed that lipoprotein deposition and hemodynamic obstruction were often found in tubulointerstitium and mesangial areas in patients with nephritis, causing high incidence of hyperlipidemia and hypocalcemia [21]. This suggested that tacrolimus was highly lipophilic and had better control of blood lipid indexes than cyclophosphamide, as well as indirectly protected kidney function by lowering blood lipid levels. Our study demonstrated that ALT showed no significant differences after treated with these two drugs. While the albumin was significantly elevated, and group A was significantly higher than group B, the Scr and BUN decreased significantly in the two groups, and group A was significantly lower than group B. Persistently decreased plasma albumin levels might lead to infection in patients with HSPN, and the long-term infection was more likely to develop into hypoalbuminemia, which was not conducive to the prognosis of patients [22]. There was a report that slower organism operation induced by reduced renal perfusion might lead to a decrease in glomerular filtration rate and an increase in Scr; meanwhile, enhanced oxidative stress triggered injuries of renal cells and tubules [23]. A study demonstrated that BUN was an end product of human protein metabo-

lism excreted mainly through glomerular filtration, and its expression level could reflect kidney injury degree [24]. According to the above references, tacrolimus is better than cyclophosphamide in improving liver and kidney function. In our findings, the IgA, IgG and IgM in the two groups were significantly decreased after treatment, and the IgA in group A was significantly lower than that in group B. Previous study showed that in patients with HSPN, the large accumulation of IgA immune complexes around glomerulus activated complements via the bypass pathway, leading to destruction of vascular endothelium, enhancement of vascular permeability, proliferation of mesangial matrix, as well as acceleration of crescent formation, and eventually immunopathologic damage of kidney [25]. It indicated that both tacrolimus and cyclophosphamide had the effect of improving the immune function of patients, avoiding the pathological changes, and enhancing the therapeutic effect, but tacrolimus was better than cyclophosphamide in patients with HSPN. It was also found in our study that the total effective rate and complication rate in group A were significantly higher than those in group B after treatment, suggesting that tacrolimus was more effective than cyclophosphamide in the treatment of HSPN, but had more potential safety concerns. The prognosis of HSPN was closely associated with the histological quantification of renal interstitial fibrosis and glomerulosclerosis [26]. Besides, tacrolimus was found to lead to a decrease in nitric oxide and vasodilators, thus shrinking renal vessels, resulting in irreversible damage to mitochondrial function after long-term oxygen deficiency, affecting renal tubular injury, and eventually leading to different degrees of interstitial fibrosis and renal tubular atrophy [27]. Moreover, another study revealed that cyclophosphamide participated in the inhibition of cellular and humoral immune responses, which helped to improve renal interstitium and avoid tissue fibrosis [28]. The validity of our experimental results was further verified by above studies.

To sum up, although both tacrolimus and cyclophosphamide are effective in treating HSPN, tacrolimus is superior to cyclophosphamide. It, however, induces more adverse reactions. Therefore, it is necessary to assess the patient's condition comprehensively in order to select the application. We explored the values of tacrolimus and cyclophosphamide by analyzing the changes in liver and kidney function and immune function of patients with HSPN. However, not all liver function indicators have changed significantly in our study, and the reasons have not been analyzed in depth, which will be explored in order to extend the application of both drugs.

Disclosure of conflict of interest

None.

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References

[1] Greco C, Iskander IF, El Houchi SZ, Rohsiswatmo R, Rundjan L, Ogala WN, Ofakunrin AOD, Moccia L, Hoi NTX, Bedogni G, Tiribelli C and Coda Zabetta CD; study team. Diagnostic performance analysis of the point-of-care bilistick system in identifying severe neonatal hyperbilirubinemia by a multi-country approach. EClinicalMedicine 2018; 1: 14-20.

[2] Hu XY, Zhang H, Rong YY, Zhang MH and Zhang XN. Clinical observation on treatment of type 2 cardiac and kidney syndrome by combination of traditional Chinese and Western medicines. Zhongguo Zhong Yao Za Zhi 2017; 42: 3815-3818.

[3] Choi JY, Ho JH, Pasoto SG, Bunin V, Kim ST, Carrasco S, Borba EF, Goncalves CR, Costa PR, Kallas EG, Bonfa E and Craft J. Circulating follicular helper-like T cells in systemic lupus erythematosus: association with disease activity. Arthritis Rheumatol 2015; 67: 988-999.

[4] Takeda S, Takahashi M, Kusano E and Kobayashi E. Mycophenolate mofetil prevents development of urinary protein in autoimmune nephritis. Kidney Int 2003; 64: 365-366; author reply 366.

[5] Hamilton P, Ogundare O, Raza A, Ponnusamy A, Gorton J, Alachkar H, Choudhury J, Barratt J and Kalra PA. Long-term therapeutic plasma exchange to prevent end-stage kidney disease in adult severe resistant Henoch-Schonlein purpura nephritis. Case Rep Nephrol 2015; 2015: 269895.

[6] Feng D, Huang WY, Hao S, Niu XL, Wang P, Wu Y and Zhu GH. A single-center analysis of Henoch-Schonlein purpura nephritis with nephrotic proteinuria in children. Pediatr Rheumatol Online J 2017; 15: 15.

[7] Sakai R, Kurasawa T, Nishi E, Kondo T, Okada Y, Shibata A, Nishimura K, Chino K, Okuyama A, Takei H, Nagasawa H and Amano K. Efficacy and safety of multitarget therapy with cyclophosphamide and tacrolimus for lupus nephritis: a prospective, single-arm, single-centre, open label pilot study in Japan. Lupus 2018; 27: 273-282.

[8] Goffin E, Devogelaer JP, Depresseux G, Squifflet JP, Pirson Y and van Yperselede de Strihou C. Evaluation of bone mineral density after renal transplantation under a tacrolimus-based immunosuppression: a pilot study. Clin Nephrol 2003; 59: 190-195.

[9] Oetjen E, Grapentin D, Blume R, Seeger M, Krause D, Eggers A and Knepel W. Regulation of human insulin gene transcription by the immunosuppressive drugs cyclosporin A and tacrolimus at concentrations that inhibit calcineurin activity and involving the transcription factor CREB. Naunyn Schmiedebergs Arch Pharmacol 2003; 367: 227-236.

[10] Dynes J, Osz K, Hooper A and Petrik J. Low-dose metronomic delivery of cyclophosphamide is less detrimental to granulosa cell viability, ovarian function, and fertility than maximum tolerated dose delivery in the mouse. Biol Reprod 2017; 97: 449-465.

[11] Huijts CM, Werter IM, Lougheed SM, Goedegebuure RS, van Herpen CM, Hamberg P, Tascilar M, Haanen JB, Verheul HM, de Gruijl TD and van der Vliet HJ; Dutch WIN-O Consortium. Phase 1 study of everolimus and low-dose oral cyclophosphamide in patients with metastatic renal cell carcinoma. Cancer Immunol Immunother 2019; 68: 319-329.

[12] Subspecialty Group of Renal Diseases, the Society of Pediatrics, Chinese Medical Association. Evidence-based guideline for diagnosis and treatment of Henoch-Schonlein purpura nephritis (2016). Zhonghua Er Ke Za Zhi 2017; 55: 647-651.

[13] Won J, Lee JS, Oh JS, Kim YG, Lee CK, Yoo B and Hong S. Impact of stringent response in proteinuria on long-term renal outcomes in proliferative lupus nephritis. Lupus 2019; 28: 1294-1301.

[14] Lopez-Mejias R, Castaneda S, Genre F, Remuzgo-Martinez S, Carmona FD, Llorca J, Blanco R, Martin J and Gonzalez-Gay MA. Genetics of immunoglobulin-A vasculitis (Henoch-Schonlein purpura): an updated review. Autoimmun Rev 2018; 17: 301-315.

[15] Qiao Y, Shin JI, Sang Y, Inker LA, Secora A, Luo S, Coresh J, Alexander GC, Jackson JW, Chang AR and Grams ME. Discontinuation of angiotensin converting enzyme inhibitors and angiotensin receptor blockers in chronic kidney disease. Mayo Clin Proc 2019; 94: 2220-2229.

[16] Lim SK and Ng FC. Acute pyelonephritis and renal abscesses in adults-correlating clinical parameters with radiological (computer tomography) severity. Ann Acad Med Singapore 2011; 40: 407-413.

[17] Hongyan L, Yi Z, Bao D, Yuewu L and Juan M. A study on clinical and pathologic features in lupus nephritis with mainly IgA deposits and a literature review. Clin Dev Immunol 2013; 2013: 289316.

[18] Kunkeler L, Nieboer C and Bruynzeel DP. Type III and type IV hypersensitivity reactions due to mitomycin C. Contact Dermatitis 2000; 42: 74-76.

[19] Erdoes G, Uehlinger DE, Kobel B, Stucki MP, Wiest R, Stueber F, Fankhauser N, Jakob SM and Schefold JC. Cerebral microembolism in the critically ill with acute kidney injury (COMET-AKI trial): study protocol for a randomized controlled clinical trial. Trials 2018; 19: 189.

[20] Hydes T, Wright M, Jaynes E and Nash K. Nitrofurantoin immune-mediated drug-induced liver injury: a serious complication of a commonly prescribed medication. BMJ Case Rep 2014; 2014.

[21] Xu XM, Zhu SS, Wang XH, Shao XF, Li B, Zhang Y, Liu Q, Li JM, Wang HL, Li YQ and Zou HQ. Clinical and pathological features in IgA nephropathy with IgG deposition in the glomerular mesangial area. Nan Fang Yi Ke Da Xue Xue Bao 2017; 37: 308-311.

[22] Lee JI, Kwon M, Roh JL, Choi JW, Choi SH, Nam SY and Kim SY. Postoperative hypoalbuminemia as a risk factor for surgical site infection after oral cancer surgery. Oral Dis 2015; 21: 178-184.

[23] Matsushita K, Saritas T, Eiwaz MB, McClellan N, Coe I, Zhu W, Ferdaus MZ, Sakai LY, McCormick JA and Hutchens MP. The acute kidney injury to chronic kidney disease transition in a mouse model of acute cardiorenal syndrome emphasizes the role of inflammation. Kidney Int 2020; 97: 95-105.

[24] Otto CM. Heartbeat: blood urea nitrogen to creatinine ratio predicts outcome in acute heart failure. Heart 2017; 103: 399-401.

[25] McClintock SD, Barron AG, Olle EW, Deogracias MP, Warner RL, Opp M and Johnson KJ. Role of interleukin-6 in immune complex induced models of vascular injury. Inflammation 2005; 29: 154-162.

[26] Xiao Y, Zhang Z, Fu Y, Shan H, Cui S and Wu J. GSTA3 regulates TGF-beta1-induced renal in-

terstitial fibrosis in NRK-52E cells as a component of the PI3K-Keap1/Nrf2 pathway. J Int Med Res 2019; 47: 5787-5801.

[27] Elens L, Bouamar R, Hesselink DA, Haufroid V, van der Heiden IP, van Gelder T and van Schaik RH. A new functional CYP3A4 intron 6 polymorphism significantly affects tacrolimus pharmacokinetics in kidney transplant recipients. Clin Chem 2011; 57: 1574-1583.

[28] Saunders P, Tsipouri V, Keir GJ, Ashby D, Flather MD, Parfrey H, Babalis D, Renzoni EA, Denton CP, Wells AU and Maher TM. Rituximab versus cyclophosphamide for the treatment of connective tissue disease-associated interstitial lung disease (RECITAL): study protocol for a randomised controlled trial. Trials 2017; 18: 275.