Serum creatinine level and ESR values associated to clinical pathology types and prognosis of patients with renal injury caused by ANCA-associated vasculitis

HONGDA LIANG*, MIAOMIAO XIN*, LEI ZHAO, LIQIN WANG, MINGSHU SUN and JIBO WANG

Department of Rheumatology and Clinical Immunology, The Affiliated Hospital of Qingdao University, Qingdao, Shandong 266005, P.R. China

Received June 20, 2017; Accepted October 5, 2017

DOI: 10.3892/etm.2017.5306

Abstract. The correlation between serum creatinine and erythrocyte sedimentation rate (ESR) values and clinical pathology and prognosis in patients with renal injury caused by anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis were analyzed. Eighty-six patients with ANCA-associated vasculitis (AAV) treated in the Affiliated Hospital of Qingdao University were enrolled in the study. Patients were assigned into an elderly group (n=45) or a non-elderly group (n=41) according to age. The serum creatinine (Scr) level was measured via the sarcosine oxidase method, and the erythrocyte sedimentation rate (ESR) was measured using the full-automatic ESR analyzer; the relationship between Scr and ESR values and the pathology type of patients was statistically analyzed. The mean levels of Scr and ESR in the 86 patients were 406.87±12.37 μmol/l and 83.83±7.64 mm/1 h, respectively. Importantly, the levels of Scr and the ESR in the elderly group were significantly higher than those in the non-elderly group (P<0.05). In addition, patients with high levels of Scr and accelerated ESR presented mainly the crescentic and sclerotic pathological types, while in the same patients the numbers of focal and mixed types were lower (P<0.05). Kaplan-Meier analysis showed that the survival rate in the elderly group was significantly lower than that in the non-elderly group, and likewise patients with high levels of Scr and accelerated ESR had significantly lower survival rates than those with low levels of Scr and normal ESR (P<0.05). The AUC of the Scr level was 0.901, the sensitivity 90.2%, the specificity 89.5% and the cut-off value was 392.5 μmol/l; while the AUC of the ESR level was 0.864, the sensitivity 89.2%, the specificity 88.5% and the cut-off value 72.8 mm/1 h. Logistic regression analysis showed that the levels of Scr (OR=2.315, P<0.01) and ESR (OR=1.847, P<0.01) were independent factors affecting the prognosis of patients. Based on our findings, the seric Scr level and the ESR are closely related to the clinicopathological features of the disease in patients with renal injury caused by ANCA-associated vasculitis, and they can be used as prognosis and treatment evaluation markers.

Correspondence to: Dr Jibo Wang, Department of Rheumatology and Clinical Immunology, The Affiliated Hospital of Qingdao University, 16 Jiangsu Road, Qingdao, Shandong 266005, P.R. China
E-mail: wangjiboqd@126.com

*Contributed equally

Key words: anti-neutrophil cytoplasmic antibody-associated vasculitis, renal injury, creatinine, erythrocyte sedimentation rate, prognosis

Introduction

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a common autoimmune disease with rapid progression, and is one of the causes of secondary kidney disease. The clinical symptoms of AAV patients are mainly fatigue, fever and emaciation (1,2). AAV often leads to multiple organ involvement, especially affecting the kidneys, altering glomerular capillaries and small arteries, leading to renal injury and causing focal necrotic glomerulonephritis, accompanied with crescent formation and renal insufficiency. Most importantly, the progression of AAV is rapid and the 5-year survival rate of patients is <60% in the absence of timely and effective renal replacement and immunosuppressive therapy (3,4). Creatinine (SCr) found in the serum is the product of muscle metabolism in the human body; it can be filtered by glomeruli and excreted in the urine. SCr is often used as a major clinical indicator of renal function (5). The erythrocyte sedimentation rate (ESR), or blood sedimentation for short, refers to the sedimentation rate of erythrocytes under certain laboratory conditions. In blood samples from a variety of pathological conditions, ESR is significantly increased, reflecting the activity of the disease to a certain extent (6). In this study, Scr levels and the ESR were measured in patients with renal injury caused by AAV, and the relationship between the values obtained and the pathology and prognosis of the patients was analyzed, so as to provide reference markers for prevention and treatment of AAV disease.

Patients and methods

General data. A total of 86 patients with AAV treated in the Affiliated Hospital of Qingdao University from December 2010 to November 2011 were enrolled in the study. Patients who

met the following conditions were included: patients diagnosed with AAV via renal biopsy and laboratory examination; patients with positive serum ANCA, accompanied by renal injury, and who had not received renal replacement therapy; and patients/gardians who signed the informed consent. Patients were excluded if they had an immune deficiency or concurrent infection; if they had used hormones in the past 3 months; or if they were suffering from cardiac insufficiency or malignant tumor. According to their age, these patients were divided into an elderly group (n=45) and a non-elderly group (n=41). Other general data differing between the patients in the two groups had no statistical significance (P>0.05) (Table I). The study was approved by the Ethics Committee of The Affiliated Hospital of Qingdao University.

Pathological examination. All patients underwent renal biopsies and took vitamin K orally 2 days before the procedure. On the day of operation, patients were put on a light liquid diet. Patients assumed a prone position, and a 10 cm-thick pillow was put under their abdomen to better expose the lower back. The patients held their breath and cooperated with the doctor during the biopsy. After the operation, the patients relaxed and lied on their back for 24 h. The tissue samples were fixed with 4% formaldehyde, and then turned into conventional paraffin-embedded sections for hematoxylin and eosin (H&E), periodic acid Schiff reaction (PAS), periodic acid-silver methenamine (PASM) and Masson trichrome stainings. An experienced pathologist observed the prepared slides under a light microscope (Leica Microsystems, Inc., Buffalo. Grove, IL, USA).

Laboratory examination. After 8 h of fasting, 5 ml venous blood samples were collected from patients. The blood samples were placed in special glass tubes with anticoagulant, and then ESRs were detected using the ESR-30 fully automatic dynamic ESR analyzer (Shanghai Xunda Medical Instrument Co., Ltd., Shanghai, China). Additionally, other 3-5 ml venous blood samples were collected from patients in the morning, and placed at room temperature for 1 h, before centrifugation at 2,053 x g for 20 min at 4˚C. Next, the supernatants were taken and stored at -80˚C. The Scr level was detected using the sarcosine oxidase method, with the relevant kits provided by Guangzhou Wondfo Biotech, and using a 7170A fully automatic biochemical analyzer (Hitachi, Japan). Relevant parameters of the assays included a temperature of 37.0˚C, with the samples placed in the test plate and detected using the two-point termination method (measuring point between 14-34, dominant wavelength of 700 nm, and sub-wavelength of 505 nm). After completing the measurements, the results were obtained via a printer automatically.

Follow-up. The patients in the two groups were followed up for 5 years, and the clinical features annotated including the end-stage renal disease characteristics (the estimated value of glomerular filtration rate <15 ml/min or dialysis) and death rates were recorded.

Evaluation criteria. The morphologic grading of ANCA-associated nephritis of Berden (7) was used to classify the pathological type of renal injury into one of four types: 1) Focal type for ≥50% normal glomeruli. 2) Crescentic type for ≥50% crescentic glomeruli. 3) Mixed type for <50% crescentic glomeruli, <50% normal glomeruli and <50% global sclerotic glomeruli. 4) sclerotic type for ≥50% global sclerotic glomeruli.

Table I. General data of objects in the study.

Item	Elderly group (n=45)	Non-elderly group (n=41)	t-value/χ^2	P-value
Sex (M/F)	21/17	19/22	0.322	0.571
BMI (kg/m^2)	20.35±2.36	20.63±2.43	0.542	0.589
MAP (mmHg)	96.87±12.37	97.35±11.58	0.185	0.854
WBC (10^9/l)	16.06±5.93	15.84±5.74	0.174	0.862
Course of disease (months)	4.03±5.83	4.14±5.35	0.091	0.928

Table II. Comparisons of Scr and ESR levels of patients in the two groups.

Group	No. of cases	Scr (μmol/l)	ESR (mm/1 h)
Elderly group	45	481.78±13.48	87.93±6.23
Non-elderly group	41	398.04±13.98	75.05±5.32
t-value		28.692	10.260
P-value		<0.001	<0.001

As mentioned above, the Scr level was detected using the sarcosine oxidase method, and the ESR level was detected using the full-automatic ESR analyzer. If the levels of Scr and ESR were higher than normal, they were designated as high. The distribution of high levels of Scr and ESR in patients with different pathological types was statistically analyzed.

Statistical analysis. The SPSS version 19.0 (SPSS Inc., Chicago, IL, USA) software was used for data processing. Measurement data were presented as mean ± standard deviation, and the t-test was used for analysis. Enumeration data were presented as rate, and analyzed via Chi-square test. The Kaplan-Meier analysis was used for survival analysis, and receiver operator characteristic (ROC) curve analysis was used for prognosis prediction. Logistic regression analysis was used for influencing factor of prognosis. A P<0.05 indicates that a given difference is statistically significant.

Results

Seric Scr levels and ESRs of the patients in the two groups. The mean levels of seric Scr and the ESR in the 86 patients were 406.87±12.37 μmol/l and 83.83±7.64 mm/1 h, respectively; with the levels of Scr and ESR in the elderly group being significantly higher than those in the non-elderly group (p<0.05) (see Table II for details).

Pathological analysis of patients and relationship between different pathological types and Scr levels and ESR. Pathology

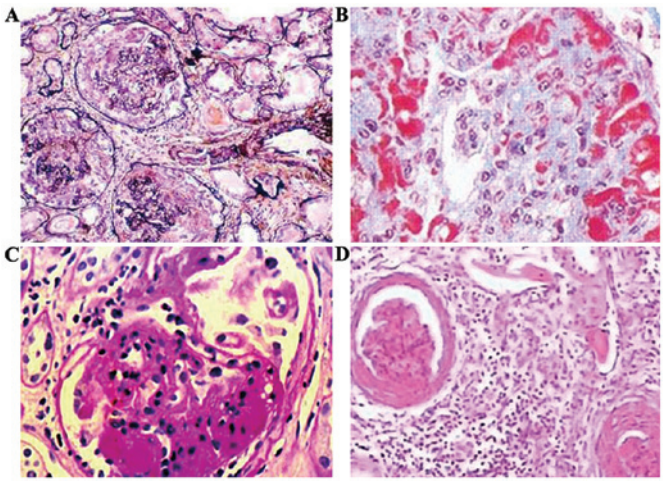

Figure 1. Light microscopic view of sample slides stained differently to reveal pathology types: (A) crescentic type after PASM staining; (B) sclerotic type after Masson staining; (C) focal type after PAS staining; (D) mixed type after hematoxylin and eosin (H&E) staining.

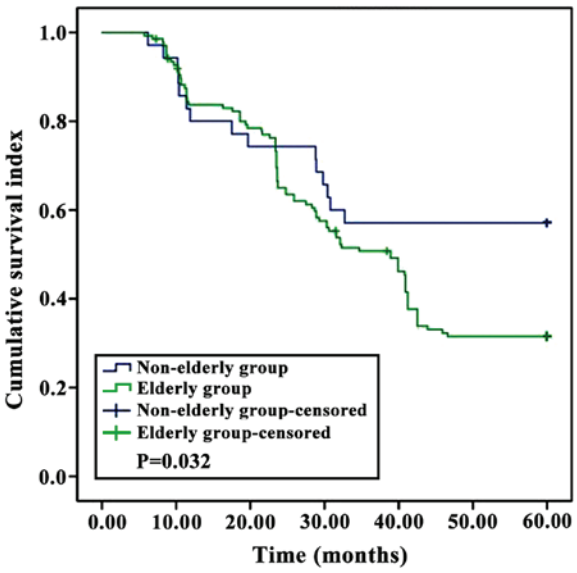

Figure 2. Kaplan-Meier analysis between elderly and non-elderly groups.

Table III. High Scr and ESR levels in patients with different pathological types (n, %).

Pathological type	No. of cases	High Scr level	High ESR level
Crescentic type	30	29 (96.67)	28 (93.33)
Sclerotic type	24	21 (87.50)	20 (83.33)
Mixed type	18	8 (44.44)	7 (38.89)
Focal type	14	5 (35.71)	4 (28.57)
χ^2		28.567	28.731
P-value		<0.001	<0.001

results showed 30 cases of crescentic, 24 of sclerotic, 18 of mixed and 14 of focal types (Fig. 1). The patients with high

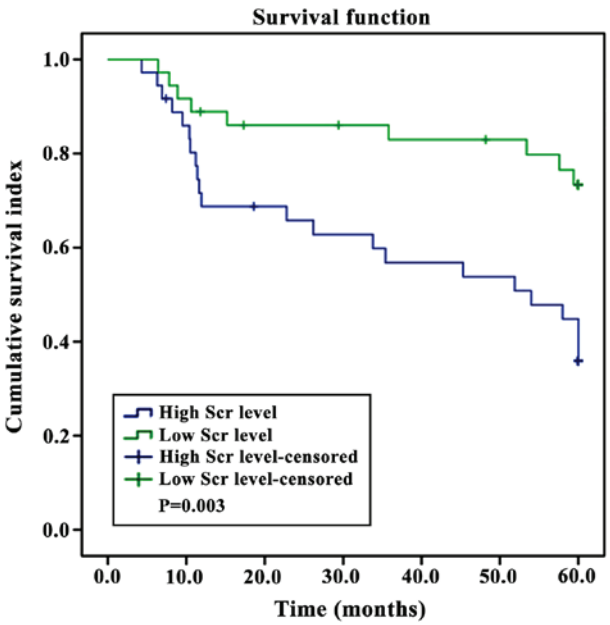

Figure 3. Kaplan-Meier analysis between high Scr and low Scr group.

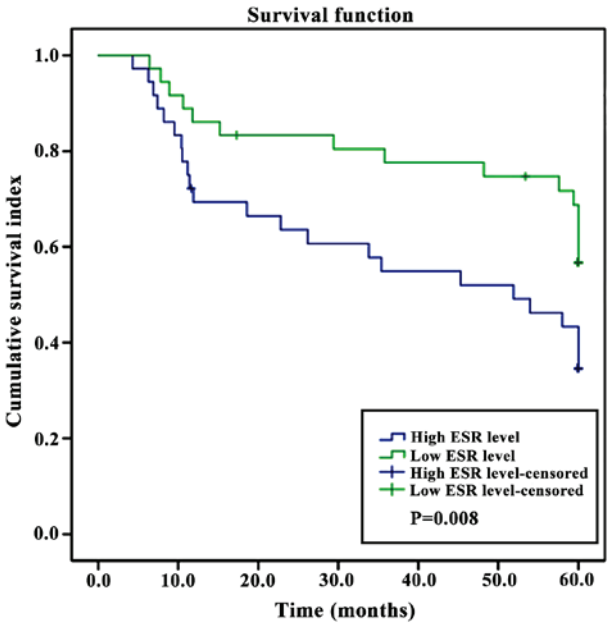

Figure 4. Kaplan-Meier analysis between high ESR and low ESR group.

levels of Scr and high ESR presented mainly the crescentic and sclerotic types, the patients in this group with focal and mixed types were significantly less (P<0.05) (Table III).

Survival rate of patients. Kaplan-Meier analysis showed that the survival rate of patients in the elderly group was significantly lower than that of patients in the non-elderly group, and the survival rate of patients with high levels of Scr and ESR was significantly lower than that of patients with low levels of Scr and ESR (P<0.05) (Figs. 2-4).

ROC analysis. The ROC analyses using the Scr and ESR values showed an area under the curve (AUC) of Scr level of 0.901,

Table IV. Logistic regression analysis of prognosis influencing factors.

Factor	β	S.E	Wald	OR	95% CI	P-value
Age	0.337	0.502	3.713	0.738	0.375-0.972	0.213
Sex	0.437	0.517	4.072	0.236	0.114-0.779	0.319
Educational level	-0.417	0.613	5.327	0.173	0.456-0.856	0.173
Course of disease	-0.615	0.824	6.405	0.237	0.196-0.512	0.218
Scr	1.426	0.749	7.757	2.315	1.475-5.252	0.004
ESR	1.433	0.517	8.524	1.847	1.113-4.347	0.003

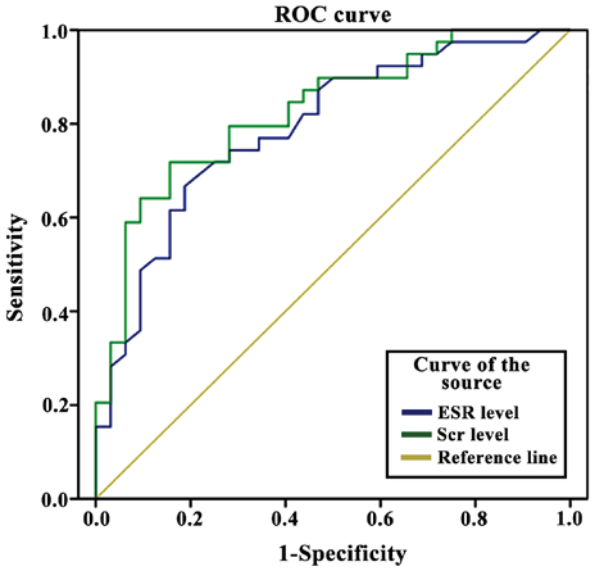

Figure 5. ROC curve analysis shows that AUC of Scr level and ESR level is 0.901 and 0.864, respectively, and both sensitivity and specificity are higher.

with 90.2% sensitivity, 89.5% specificity, and a cut-off value of 392.5 μmol/l; while the AUC of the ESR value was 0.864, with a sensitivity of 89.2%, a specificity of 88.5% and a cut-off value of 72.8 mm/1 h (Fig. 5).

Analysis of prognosis influencing factors. With poor prognosis as the dependent variable and age, sex, educational level, course of disease, Scr and ESR values as independent variables, the logistic regression analysis performed showed that Scr (OR=2.315, P=0.004) and ESR (OR=1.847, P=0.003) were independent risk factors affecting the poor prognosis of patients (P<0.05) (Table IV).

Discussion

ANCA-associated vasculitis (AAV) is a kind of autoimmune disease occurring commonly in the elderly; and its pathological characteristics include necrotic inflammation in vascular walls and abnormal death of neutrophils. AAV belongs to the small-vessel vasculitis group of systemic vasculitis, with positive ANCA antibodies in serum and a lack of immune complex deposition in vascular walls (8,9). ANCA is an autoantibody with monocyte lysosomal components and PMN cytoplasmic particles as special antigens (immunoglobulin-like) (10). At present, the detection methods for ANCA include mainly the enzyme-linked immunosorbent assay (ELISA) and the indirect immunofluorescence methods. ANCA detection was incorporated into the standardized detection system in the 1990s in China, so the detection rate of AAV has been increasing ever since. The pathogenesis of AAV remains unclear. However, many factors have been implicated in the development of the disease. Genetic factors include the CD226-encoded antigen, the HLA gene (HLA-DR4 and HLA-DR13), the PTPN22 protein and the IL-I0 gene, which can participate in the immune mechanisms of AAV.

Moreover, environmental factors such as hydrocarbons, silica dust, microbial (*Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*) infections and drugs (hydralazine, propylthiouracil, minocyline and penicillamine) have been associated with AAV (11). A general route for the development of AAV would theoretically begin with environmental factors stimulating inflammatory mediators (TNF-α, IL-10 and IL-8); which would, in turn, activate PMN cells to express antigen components (PR3 and MPO) onto their cell surface, and get combined with ANCAs, leading to the full activation of PMN. During the process of PMN activation, the expression of adhesion molecules is increased, so the PMN and endothelial cells contact each other closely; which results in various inflammatory mediators, lysosomal enzymes and toxic oxygen free radicals play a direct cytotoxic effect, causing vascular endothelial injury in multiple organ sites, especially the in the kidneys (12). Clinical symptoms of patients with renal injury caused by AAV include oliguria, anuria, decreased renal function or acute oliguric renal failure. If there is no timely treatment, AAV develops rapidly and ultimately leads to irreversible end-stage renal failure (13).

A clinical treatment method for patients with AAV-related renal injury consists often of a glucocorticoid (methylprednisolone) combined with cytotoxic drugs (14). Clinically, renal biopsy is often used to diagnose AAV-related renal injury and evaluate the prognosis. But high-sensitivity and high-specificity markers for early diagnosis and prognosis evaluation would be preferable due to the invasive nature of the renal biopsy procedure (15,16).

Scr is the final metabolite of phosphocreatine via a non-enzymatic dehydration reaction. Scr is excreted by glomeruli but not reabsorbed by renal tubules. The normal seric level of Scr ranges from 44 to 133 μmol/l, and high levels can reflect the severity of glomerular injury (17,18).

ESR, on the other hand, is a non-specific marker that reflects well the status of the AAV disease. The acceleration

of the ESR is often associated with a variety of diseases, such as acute bacterial inflammation, active tuberculosis, nephritis, myocarditis, pneumonia, rheumatoid arthritis, systemic lupus erythematosus, and others; and tissue damage and necrosis also accelerate the ESR (19,20). The results of this study showed that the mean level of Scr and the ESR in 86 patients was, respectively, 406.87±12.37 μmol/l and 83.83±7.64 mm/h. Additionally, the levels of Scr and the ESR of patients in the elderly group were significantly higher than those of patient in the non-elderly group ($P<0.05$). This is because renal injury leads to decreased glomerular filtration function and changes in the glomerular filtration rate; so the concentration of Scr is increased, and with the worsening of the disease, the ESR is significantly accelerated.

The pathology results in this study showed 30 cases of crescentic type, 24 cases of sclerotic type, 18 cases of mixed type and 14 cases of focal type. The number of patients with high Scr and ESR values was significantly higher in those patients with crescentic and sclerotic types than in patients with focal and mixed types. This may be due to the fact that patients of focal type have a more stable renal function, and the patients with the mixed types generally have a more benign renal disease; while the patients with sclerotic type cannot have their renal function restored and those with crescentic type have also a more serious alteration of their kidneys.

Different renal outcomes are closely related to the survival and life quality of patients affected with AAV. Treatment is aimed at improving the long-term survival and life quality of patients. Kaplan-Meier analysis showed that the survival rate of patients in the elderly group was significantly lower than that of patients in the non-elderly group. The survival rate of patients with high Scr and ESR values was significantly lower than that of patients with low Scr and ESR values. Furthermore, the ROC curve test showed Scr levels and the ESR should be very useful in diagnosis and prognosis of AAV, and logistic regression analysis showed that high Scr levels and an accelerated ESR were independent risk factors each leading to poor prognosis. These findings suggest that the levels of Scr and the ESR values can reflect the severity of renal injury to a certain extent and help in understand the activity, remission or relapse condition of the kidney disease. The changes in the two markers should be monitored during clinical treatment and follow-up, so as to take effective intervention measures to improve the prognosis of patients with AAV. However, this study was limited by a small sample size, so long-term large-sample studies are still needed before issuing precise recommendation.

In conclusion, monitoring the serum level of Scr and the ESR value in patients with renal injury by AAV can help practitioners prescribe interventions that may alter the course of the disease and improve the prognosis for the patients.

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