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Association of serum high mobility group box 1 levels with disease activity and renal involvement in patients with systemic vasculitis

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Abstract

High mobility group box 1 (HMGB1) is a kind of proinflammatory mediator that acts as an alarmin when released by dying, injured or activated cells. Previous studies have reported that HMGB1 are closely linked to antineutrophil cytoplasmic antibody-associated vasculitis (AAV). The present study aimed to evaluate whether serum HMGB1 levels were associated with systemic vasculitis (VAs).

The study population consisted of 51 patients with VAs, 46 patients with essential hypertension (EH) and 46 healthy controls (HC). Thirty-five patients with VAs had in active stage and 16 patients with VAs in an inactive stage. Furthermore, 31 patients with VAs had renal involvement, the other 20 patients were selected for without renal involvement. Serum HMGB1 levels were measured by enzyme-linked immunosorbent assay. Associations between serum HMGB1 levels with clinical and laboratory parameters were analyzed.

Serum HMGB1 levels in patients with VAs were significantly higher than in EH and HC (all $P < .05$), and no difference regarding serum HMGB1 levels could be found between EH and HC ($P = .208$). Serum HMGB1 levels in VAs patients with active stage were significantly higher than those in HC and VAs patients with inactive stage (all $P < .05$). Patients with renal involvement and non-renal involvement had increased HMGB1 levels compared with HC (all $P < .05$). In addition, serum HMGB1 levels were significantly higher in patients with renal involvement compared with non-renal involvement patients ($P = .001$). Correlation analysis showed that serum HMGB1 levels were positive significant correlated with the Birmingham Vasculitis Activity Score, hypersensitive C reactive protein (Hs-CRP), serum creatinine (Scr) and 24-hour proteinuria (all $P < .05$). Among the subsets of VAs, serum HMGB1 levels were significantly higher in AAV, polyarteritis nodosa (PAN) and takayasu arteritis (TA) than in HC (all $P < .05$). More interestingly, serum HMGB1 were significantly higher in patients with PAN compared with AAV and TA patients (all $P < .05$). Furthermore, there was positive correlation between serum HMGB1 levels and Hs-CRP, Scr, and 24-hour proteinuria in patients with PAN (all $P < .05$).

Serum HMGB1 levels are increased in patients with VAs compared with HC and EH and can reflect the disease activity and renal involvement.

Abbreviations: AAV = antineutrophil cytoplasmic antibody-associated vasculitis, BVAS = Birmingham Vasculitis Activity Score, EH = essential hypertension, ELISA = enzyme-linked immunosorbent assay, GPA = granulomatosis with polyangiitis, HC = healthy controls, HMGB1 = high mobility group box 1, Hs-CRP = hypersensitive C reactive protein, PAN = polyarteritis nodosa, Scr = serum creatinine, TA = takayasu arteritis, VAs = systemic vasculitis.

Keywords: biomarker, high mobility group box 1, polyarteritis nodosa, systemic vasculitis, takayasu arteritis

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All the participants to the present study had signed the consent form before participation. The study protocol was approved by the ethics committee of the People's Hospital of Xinjiang Uygur Autonomous Region.

The authors declare that they have no competing interests.

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1. Introduction

Systemic vasculitis (VAs) is a group of complex, chronic, and potentially disabling diseases characterized by inflammation damage and destruction that affect all types of size vessels, responsible for marked morbidity and societal burden.[1–4] Thus far, the underlying mechanism is not fully clear yet. Because of the location, type, and range of the involved vessels, the clinical manifestations vary greatly, and can occur in patients of every age.[5] Therefore, the diagnosis and disease assessment of VAs is very difficult.

High mobility group box 1 (HMGB1) is a group of non-histone nuclear protein by being actively secreted from activated immune cells or passively released from injured or dying cells and becomes a proinflammatory mediator via binding to various receptors (such as RAGE, TLR-2, TLR-4, and TLR-9) on the surface of responding cells.[6] HMGB1 acts as a damage-associated molecular pattern or a so-called alarmin to stimulate the innate and adaptive immune system and participates in all kinds of acute and chronic inflammatory process after sterile injury or microbial invasion.[7–8] HMGB1 has been suggested to be involved in the pathogenesis of many autoimmune diseases.[9–12] In VAs, recent studies have shown that high serum HMGB1 levels were observed in kawasaki disease and antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) patients, especially in granulomatosis with polyangiitis (GPA) with granulomatous manifestations.[13–16] However, other studies showed that patients with AAV, takayasu arteritis (TA) and giant cell arteritis present similar serum HMGB1 levels compared with healthy controls (HC).[17,18] Therefore, whether HMGB1 as a kind of proinflammatory mediator is associated with VAs is still controversial, and studies are scarce especially in polyarteritis nodosa (PAN). In this study, we measured serum HMGB-1 levels in VAs patients and evaluate whether serum HMGB1 levels were associated with VAs, and correlated serum HMGB1 levels with clinical and laboratory parameters.

2. Methods

2.1. Patients

The study consecutively selected the 51 patients with VAs (including 20 AAV, 24 PAN, and 7 TA) diagnosed at the Center for Hypertension of People's Hospital of Xinjiang Uygur Autonomous Region from January 2013 and December 2017. All these VAs patients were consulted by the rheumatology doctor and fulfilled of the 1990 American College of Rheumatology and/or the 2012 revised International Chapel Hill Consensus Conference classification criteria.[19–23] The diagnostic flowchart of VAs was shown in Figure 1. Disease activity was assessed in accordance with the third version of Birmingham Vasculitis Activity Score (BVAS).[24] Patients with BVAS ≥ 1 were considered active stage, and patients with a BVAS score $= 0$ were considered to have the inactive stage. Patients with secondary VAs, systemic lupus erythematosus, rheumatoid arthritis, malignancy, infection, or with any other coexisting renal disease, such as antiglomerular basement membrane nephritis, IgA nephropathy, diabetic nephropathy, or lupus nephritis were excluded.

Essential hypertension (EH) patients were recruited from the Center for Hypertension of People's Hospital of Xinjiang Uygur Autonomous Region after excluding those with evidence of secondary hypertension, any acute or active chronic infection, diabetes and nephropathy by clinical examination, and eventually 46 EH were included. HC were recruited from the Center for Medical examination of the People's Hospital of Xinjiang Uygur Autonomous Region after excluding those with evidence of any acute or chronic infection as well as diseases that cause vascular damage, such as hypertension, diabetes, and nephropathy by clinical examination, and eventually 46 HC were included.

All the participants to the current study had signed the consent form before participation. The study protocol was approved by the ethics committee of the People's Hospital of Xinjiang Uygur Autonomous Region.

2.2. Data collection and measurements

All the information of clinical data came from the patient's medical records during hospitalization (including demographics, clinical, biologic, imaging, and biopsy findings).

The following clinical manifestations were recorded: general symptoms (fever, weakness, asitia, and weight loss); myalgias and arthralgias; decreased brachial artery pulse, difference of >10 mm Hg in systolic blood pressure between arms, and bruit from subclavian arteries or abdominal aorta; peripheral neuropathy (mononeuritis multiplex or polyneuropathy); central nervous system involvement; urologic and renal involvement (orchitis, dialysis, peripheral limb edema, and recent-onset or severe hypertension); cutaneous symptoms (nodules, purpura, erythra, and livedo); alimentary manifestations (nausea, vomiting abdominal pain, hemorrhage, pancreatitis, and peritonitis); cardiovascular involvement (pectoralgia, cardiomyopathy, pericarditis, and ischemic); ophthalmologic involvement (retinal VAs/exudates, visual impairment, conjunctivitis, keratitis, and uveitis); pulmonary involvement (cough, hemoptysis, dyspnea, pleural effusion, and lung infiltrates).

Biologic parameters: blood cell counts; renal parameters (proteinuria, hematuria, 24-hour proteinuria, and serum creatinine (Scr)); erythrocyte sedimentation rate; C-reactive protein (CRP); hypersensitive C-reactive protein (Hs-CRP); and the ANCA testing by indirect immunofluorescence and enzyme-linked immunosorbent assay (ELISA).

Imaging examination: the angiographies results as abnormal when showing the blood vessels was sparse, irregular stenoses and/or microaneurysms; chest X-ray showed that nodules, infiltrating lesions and/or cavity. The result was determined by 2 radiologists.

Biopsy findings: inflammatory cell infiltration was present in small- and medium-vessel and/or formation of crescent; immunofluorescence demonstrated that no or little immune complex deposition in the mesangial area, vascular loops or small vascular walls. The result was determined by 2 pathologists.

2.3. Definitions of renal injury

Renal injury was defined as the presence of any hematuria and/or proteinuria and/or Scr increased. Hematuria was defined as more than 5 red blood cells per high-power field in urine sediment. Proteinuria was defined as more than 1+ in urine routine and/or 24-hour urine collection containing more than 150 mg of proteins and was considered nephrotic when ≥ 50 mg/kg/day. Scr increased was defined as male Scr >104 μmol/L or female Scr >84 μmol/L.

2.4. ELISA for serum HMGB1

The blood samples of all the participants were drawn into procoagulation tubes. The serum was collected immediately

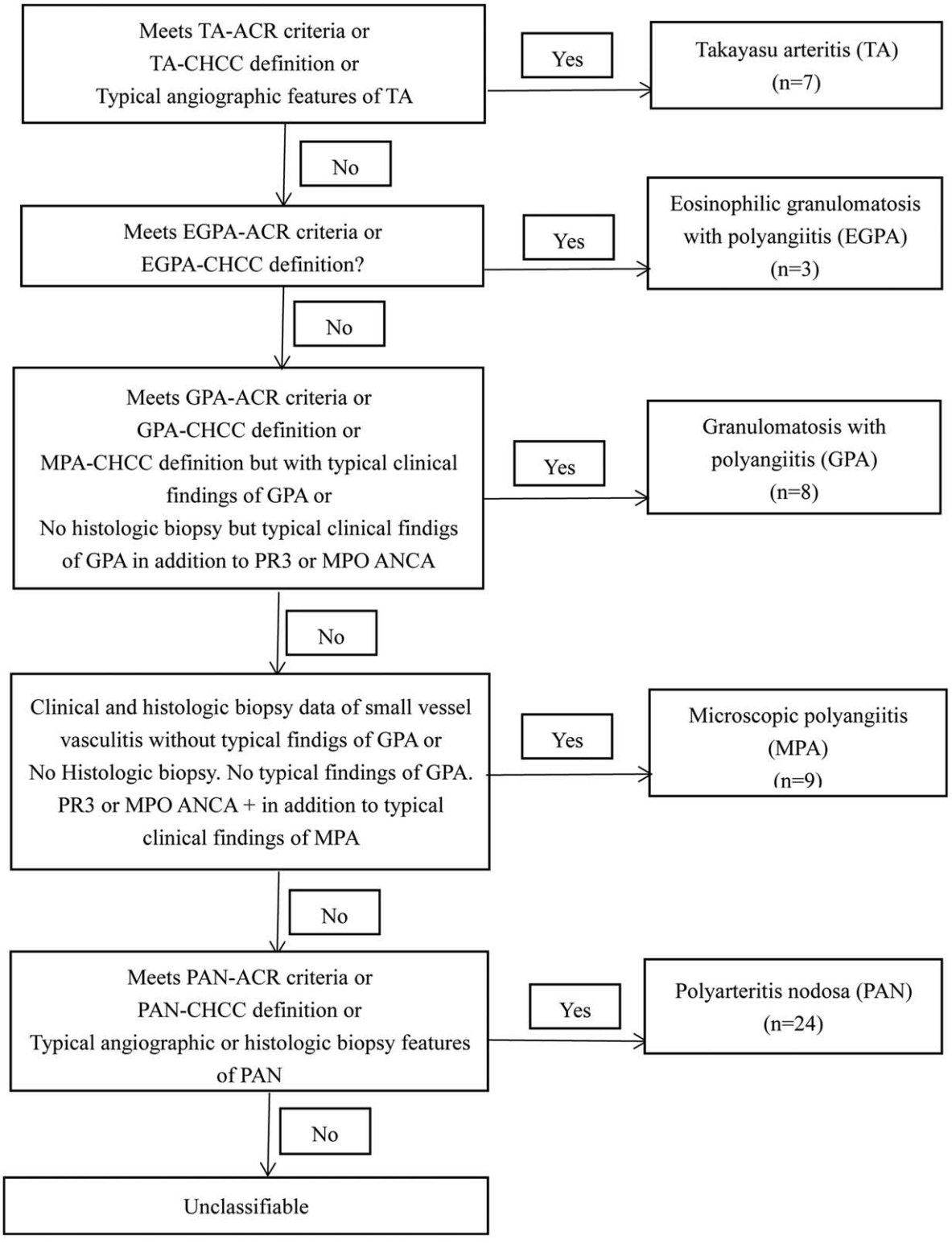

Figure 1. The criteria of diagnosis of systemic vasculitis. ACR = American College of Rheumatology, ANCA = antineutrophil cytoplasmic antibody, CHCC = Chapel Hill Consensus Conference, EGPA = eosinophilic granulomatosis with polyangiitis, GPA = granulomatosis with polyangiitis, MPA = microscopic polyangiitis, MPO = myeloperoxidase, PAN = polyarteritis nodosa, PR3 = proteinase 3, TA = takayasu arteritis.

after centrifugation at 3000g for 15 minutes at 4°C. Then the serum samples were stored at −80°C until tested. Serum HMGB1 levels were assessed with patients in VAs, EH, and HC using a commercial ELISA kit according to the manufacturer's instructions (Uscn Life Science Inc, Wuhan, China). Serum samples were diluted 1:100. Results of serum HMGB1 levels are expressed in nanograms per milliliter.

Table 1

Demographic and laboratory features of patients with VAs, EH, and HC.

Variables	VAs (n = 51)	EH (n = 46)	HC (n = 46)	*P*-value	AAV (n = 20)	PAN (n = 24)	TA (n = 7)
Demographic features							
Age, yr	40.22 ± 9.62	43.74 ± 9.22	42.61 ± 15.87	.287	42.50 ± 8.78	38.54 ± 9.45	38.00 ± 12.29
Females, n (%)	22 (43.1)	15 (32.6)	26 (56.5)	.121	11 (55.0)	7 (29.2)	4 (57.1)
SBP, mm Hg	164.65 ± 27.33	142.50 ± 17.59*	118.26 ± 11.34*	<.001	161.95 ± 29.09	167.08±26.16	164.00 ± 29.54
DBP, mm Hg	104.20 ± 19.26	88.11 ± 11.31*	73.13 ± 8.55*	<.001	99.05 ± 24.10	110.00 ± 14.59	99.00 ± 13.40
Laboratory features							
ESR, mm/h	18.43 ± 14.21	10.22 ± 7.77*	9.23 ± 5.15*	.003	20.80 ± 15.43	15.25 ± 15.95	22.57 ± 14.41
Scr, umol/L	106.62 ± 45.43	66.33 ±16.56*	72.36 ± 12.44*	<.001	107.33 ± 55.64	108.79 ± 38.51	97.14 ± 43.30
WBC, $\times 10^9$/L	7.37 ± 2.83	6.22 ± 1.43*	6.53 ± 1.83	.026	6.70 ± 2.79	7.33 ± 1.80	9.42 ± 4.85
HB, g/L	138.59 ± 20.95	145.17 ± 11.99	150.38 ± 15.18*	.015	134.35 ± 25.09	145.58 ± 15.96	126.71 ± 16.31
PLT, ×109/L	245.10 ± 106.73	254.65 ± 67.74	262.50 ± 44.85	.552	252.40 ± 95.19	222.63 ± 80.71	301.29 ± 188.09
ANCA (+), n (%)	8 (15.7)	0 (0)*	0 (0)*	<.001	7 (35.0)	0 (0)	1 (14.3)
Hs-CRP, mg/L	2.82 (0.91–8.35)	1.04 (0.42–2.64)*	—	.001	2.64 (0.77–7.72)	2.97 (1.04–8.17)	5.59 (0.72–26.99)
Proteinuria (+), n (%)	20 (39.2)	0 (0)*	0 (0)*	<.001	7 (35.0)	11 (45.8)	2 (28.6)
Hematuria (+), n (%)	7 (13.7)	0 (0)*	0 (0)*	<.001	3 (15.0)	3 (12.5)	1 (14.3)
24-h proteinuria, g	0.20 (0.06–0.61)	0.06 (0.04–0.09)*	—	<.001	0.12 (0.05–0.37)	0.28 (0.12–0.71)	0.23 (0.03–0.79)
HMGB1, ng/ml	27.20 ± 12.24	16.27 ± 8.18*	13.77 ± 6.68*	<.001	23.13 ± 10.27	32.49 ± 13.24	20.71 ± 5.12

Continuous variables are presented as median and interquartile range or as mean ± standard deviation.

AAV = ANCA-associated vasculitis, ANCA = antineutrophil cytoplasmic antibody, DBP = diastolic blood pressure, ESR = erythrocyte sedimentation rate, HB = hemoglobin, HC = healthy controls, HMGB1 = high mobility group box 1, Hs-CRP = hypersensitive C-reactive protein, PAN = polyarteritis nodosa, PLT = platelet, SBP = systolic blood pressure, Scr = serum creatinine, TA = takayasu arteritis,VAs = systemic vasculitis, WBC = white blood cell.

* Compared with group VAs, $P < .05$.

2.5. Statistical analysis

Statistical analysis was performed using SPSS software version 20.0 and graphs were built using GraphPad Prism version 5.0. Mean ± standard deviation or median and interquartile range were used to present normally distributed and non-normally distributed continuous variables, respectively. Categorical variables were presented as total number and percentage. A Student *t* test or a Mann–Whitney *U* test was used for comparison of different groups as appropriate. Spearman or Pearson rank correlation was used to assess correlations. Multiple logistic regression analysis was used to identify the independent predictor of VAs, and the odds ratios with 95% confidence intervals (CI) were calculated. Receiver operating characteristic (ROC) curve analysis was used to identify optimal cut-off values of HMGB1.[25] The cutoff value was chosen from the maximized sum of sensitivity and specificity. In addition, to further improve clinical sensitivity or specificity, multiple biomarkers were used for combined diagnosis, binary logistic regression analysis and ROC curves were analyzed. *P*-value < .05 was considered significant.

3. Results

3.1. Clinical and laboratory features of VAs patients, EH, and HC

Among the 51 patients with VAs, 29 were male and 22 were female, and the mean age at this study entry was 40.02 years. Demographic features were similar in the EH (31 male and 15 female with the mean age was 43.74 years) and HC (20 male and 26 female with the mean age was 42.61 years). 20 VAs patients were diagnosed as AAV, 24 patients were diagnosed as PAN and the other 7 were diagnosed as TA. In addition, 35 patients with VAs in active stage and 16 patients with VAs in an inactive stage. Thirty-one patients with VAs had renal involvement, the other 20 VAs patients were selected for having without renal involvement. Clinical and laboratory features of the 51 VAs patients, 46 EH, and 46 HC included in the study are presented in Tables 1 and 2.

3.2. Serum HMGB1 levels by ELISA

HMGB1 levels in serum samples from patients with VAs, EH, and HC were assessed using a commercial ELISA kit. Serum HMGB1 levels in patients with VAs were significantly higher compared to EH and HC (VAs vs EH: [27.20 ± 12.24] vs [16.27 ± 8.18] ng/ml, $P < .001$; VAs vs HC: [27.20 ± 12.24] vs [13.77 ± 6.68] ng/ml, $P < .001$) (Fig. 2A). No significant differences in serum HMGB1 levels were observed between EH and HC ([16.27 ± 8.18] vs [13.77 ± 6.68] ng/ml, $P = .208$) (Fig. 2A).

Compared to HC, patients with active stage showed the highest levels of serum HMGB1 ([30.33 ± 12.41] vs [13.77 ± 6.68] ng/ml, $P < .001$), followed by that of patients with inactive stage ([20.36 ± 8.79] vs [13.77 ± 6.68] ng/ml, $P = .003$) (Fig. 2B). Furthermore, serum HMGB1 levels were significantly higher in patients with active stage than in those with inactive stage ([30.33 ± 12.41] vs [20.36 ± 8.79] ng/ml, $P = .006$) (Fig. 2B).

VAs patients with renal involvement and non-renal involvement had increased HMGB1 levels compared with HC, the differences were statistically significant (Renal vs HC: [31.43 ± 12.11] vs [13.77 ± 6.68] ng/ml, $P < .001$; Non-renal vs HC: [20.65 ± 9.41] vs [13.77 ± 6.68] ng/ml, $P = .006$) (Fig. 2C). In addition, serum HMGB1 levels were significantly higher in patients with renal involvement compared with non-renal involvement patients ([31.43 ± 12.11] vs [20.65 ± 9.41] ng/ml, $P = .001$) (Fig. 2C).

Among the subsets of VAs, serum HMGB1 levels were significantly higher in AAV, PAN, and TA than in HC (AAV vs HC: [23.13 ± 10.27] vs [13.77 ± 6.68] ng/ml, $P < .001$; PAN vs HC: [32.49 ± 13.24] vs [13.77 ± 6.68] ng/ml, $P < .001$; TA vs HC: [20.71 ± 5.12] vs [13.77 ± 6.68] ng/ml, $P = 0.012$). More interestingly, serum HMGB1 was significantly higher in patients with PAN compared with AAV and TA patients (PAN vs AAV: [32.49 ± 13.24] vs [23.13 ± 10.27] ng/ml, $P = .009$; PAN vs TA: [32.49 ± 13.24] vs [20.71 ± 5.12] ng/ml, $P = .020$) (Fig. 2D). There was no significant difference in serum HMGB1 levels between AAV and TA ([23.13 ± 10.27] vs [20.71 ± 5.12] ng/ml, $P = .630$) (Fig. 2D).

Table 2

Clinical features of patients with systemic vasculitis.

Clinical features	VAs (n = 51)	AAV (n = 20)	PAN (n = 24)	TA (n = 7)
Headache, n (%)	30 (58.8)	9 (45.0)	18 (75.0)	3 (42.9)
Constitutional symptoms, n (%)	32 (62.7)	13 (65.0)	15 (62.5)	4 (57.1)
Nervous systems, n (%)	4 (7.8)	2 (10.0)	1 (4.2)	1 (14.3)
Renal systems, n (%)	31 (60.8)	12 (60.0)	16 (66.6)	3 (42.9)
Cutaneous, n (%)	4 (7.8)	0 (0)	3 (12.5)	1 (14.3)
Arthritis/joint pain, n (%)	2 (3.9)	0 (0)	2 (8.3)	0 (0)
Eye, n (%)	15 (29.4)	7 (35.0)	7 (29.2)	1 (14.3)
Ear nose throat, n (%)	0 (0)	0 (0)	0 (0)	0 (0)
Pulmonary system, n (%)	9 (17.6)	9 (45.0)	0 (0)	0 (0)
Alimentary system, n (%)	6 (11.8)	3 (15.0)	2 (8.3)	1 (14.3)
Cardiovascular system, n (%)	10 (19.6)	5 (25.0)	5 (20.8)	0 (0)
Active disease, n (%)	35 (68.6)	13 (65.0)	19 (79.2)	3 (42.9)
BVAS	11.35 ± 8.61	12.35 ± 10.82	11.21 ± 6.36	9.00 ± 9.07

Continuous variables are presented as median and interquartile range or as mean ± standard deviation.

AAV = antineutrophil cytoplasmic antibody-associated vasculitis, BVAS = Birmingham Vasculitis Activity Score, PAN = polyarteritis nodosa, TA = takayasu arteritis, VAs = systemic vasculitis.

3.3. Correlations of serum HMGB1 levels with clinical and laboratory parameters of patients with VAs

We evaluated whether serum levels of HMGB1 are in correlation with clinical and laboratory parameters in VAs patients. The correlation analysis showed that serum HMGB1 levels were positive significant correlated with BAVS ($r = 0.388$, $P = .005$), Hs-CRP ($r = 0.336$, $P = .016$), Scr ($r = 0.570$, $P < .001$), and 24-hour proteinuria ($r = 0.391$, $P = .005$) (Fig. 3). Furthermore, we investigated the association between serum HMGB1 levels and clinical, laboratory parameters in patients with PAN. The results showed that there was a significant correlation between serum HMGB1 levels and Scr ($r = 0.676$, $P < .001$), 24-hour proteinuria ($r = 0.456$, $P = .025$). Furthermore, multivariate

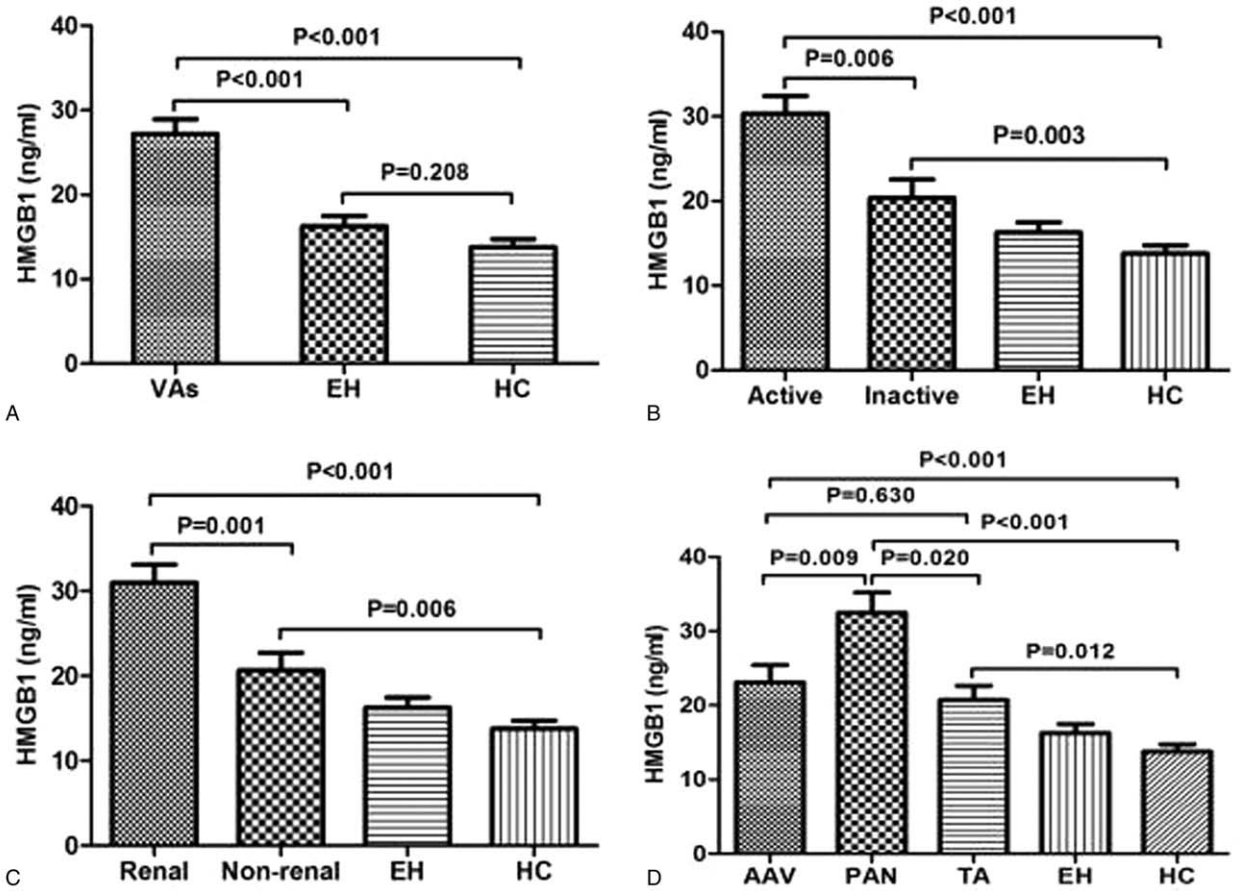

Figure 2. Serum HMGB1 levels in different groups. A: Serum HMGB1 levels in patients with systemic VAs and controls. B: Serum HMGB1 levels in VAs patients with the active stage and inactive stage. C: Serum HMGB1 levels in VAs patients with renal involvement and without renal involvement. D: Serum HMGB1 levels in VAs subsets. HMGB1 = high-mobility group box 1, VAs = systemic vasculitis.

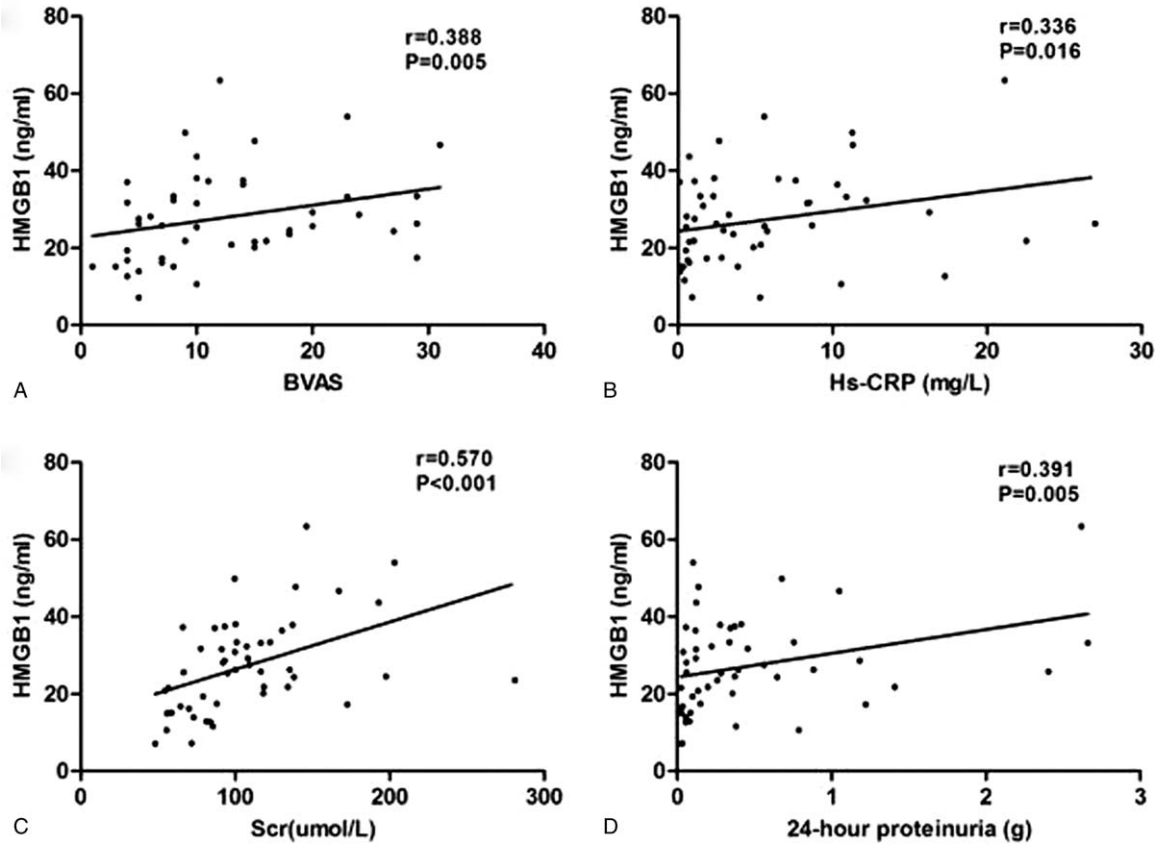

Figure 3. Correlations of HMGB1 levels with BVAS, Hs-CRP, Scr and 24-h proteinuria in patients with systemic vasculitis. BVAS = Birmingham Vasculitis Activity Score, HMGB1 = high-mobility group box 1, Hs-CRP = hypersensitive C-reactive protein, Scr = serum creatinine.

logistic regression analysis showed that HMGB1 and Scr were all independently associated with VAs (Table 3).

3.4. ROC curve analysis was used to identify optimal cutoff values of serum HMGB1

The best cutoff value of serum HMGB1 levels were estimated by calculating ROC curves. For the diagnosis of VAs, the best cutoff value was at 16.10 ng/ml. The sensitivity and specificity were calculated as 80.4% and 71.7% (Fig. 4A). ROC analysis of all patients with active stage and renal involvement showed that the 21.76 and 21.65 ng/ml were identified as the best cutoff value for serum HMGB1 levels, resulting in a sensitivity of 77.1%, 83.9%, and a specificity of 62.5% and 65.0% for VAs (Fig. 4B and C). Furthermore, ROC analysis of patients with VAs subsets showed that the best HMGB1 cutoff value for differentiating PAN from VAs is 26.27 ng/ml with 70.8% sensitivity and 77.9% specificity (Fig. 4D). All estimated values were shown in Table 4. Furthermore, it will be much more informative to test the power of HMGB1 and other known biomarkers (or correlated biomarkers) in classifying the clinical outcomes of VAs. Binary logistic regression and ROC curves explored biomarker combinations, which the combination of biomarkers was HMGB1 and Scr. Among the VAs patients, after adjusting by the regression coefficient of the binary logistic regression, the combination of HMGB1 and Scr had a sensitivity of 72.5% and a specificity of 91.3% (AUC = 0.86, 95% CI = 0.80–0.93) (Fig. 4E). For the VAs patients with active, after adjusting by the regression coefficient of the binary logistic regression, the combination of HMGB1 and Scr had a sensitivity of 77.1% and a specificity of 75.0% (AUC = 0.81, 95% CI = 0.69–0.93) (Fig. 4F).

Table 3

Logistic regression analysis of patients with systemic vasculitis versus controls.

	Multivariate		
Variable	**OR**	**95% CI**	***P*-value**
Age	0.942	0.877–1.011	.098
Sex	0.178	0.027–1.171	.073
SBP	1.007	0.999–1.015	.077
BMI	0.865	0.710–1.054	.150
WBC	0.936	0.573–1.528	.791
HB	0.971	0.913–1.033	.356
PLT	1.000	0.987–1.103	.997
Hs-CRP	1.251	0.944–1.657	.119
ESR	1.022	0.922–1.132	.679
Scr	1.109	1.039–1.184	.002
HMGB1	1.056	1.010–1.105	.016

BMI = body mass index, CI = confidence intervals, ESR = erythrocyte sedimentation rate, HB = hemoglobin, HMGB1 = high mobility group box 1, Hs-CRP = hypersensitive C-reactive protein, OR = odds ratio, PLT = platelet, SBP = systolic blood pressure, Scr = serum creatinine, VAs = systemic vasculitis, WBC = white blood cell.

4. Discussion

VAs is a kind of autoimmune disease with insidious onset, multiple organ, and systemic damage. Because of the disease course is protracted and silent relapses are common. It is not easy

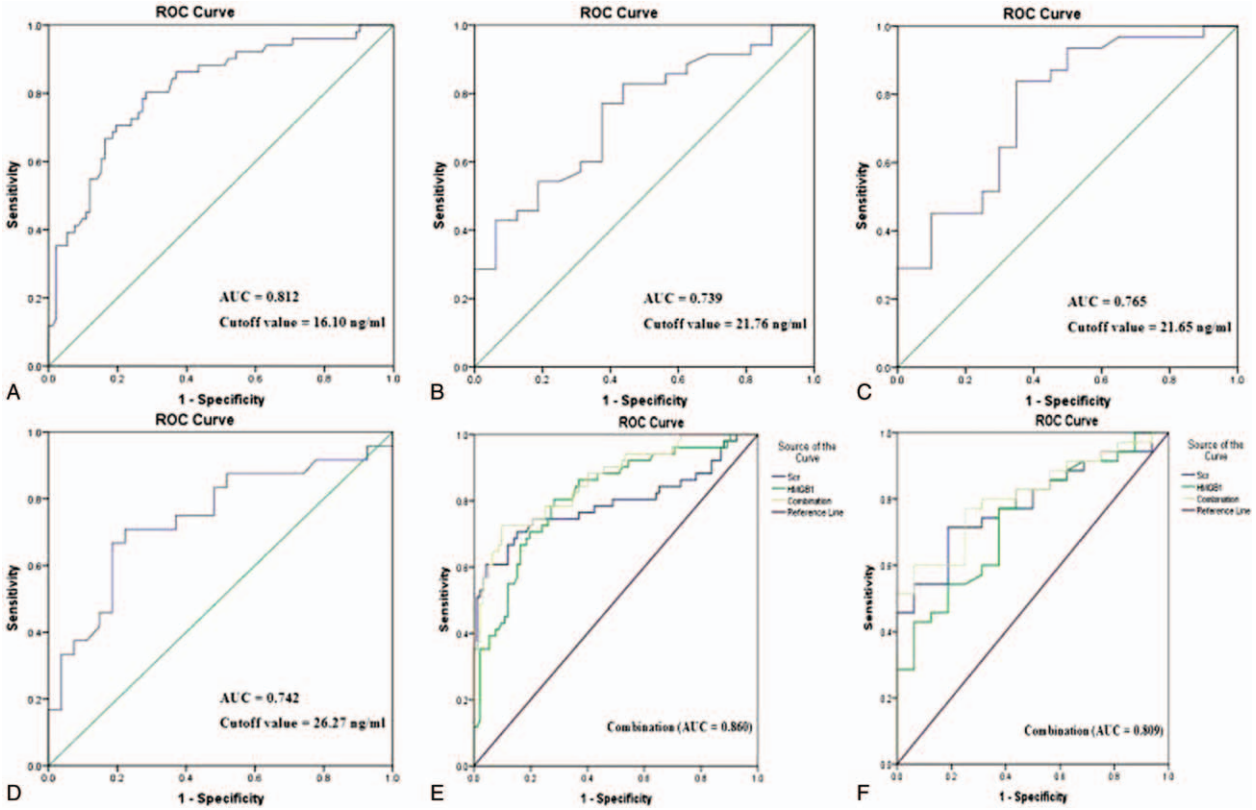

Figure 4. The ROC curves for diagnosis in different groups. A: ROC curves for diagnosis between patients with systemic VAs and controls using serum HMGB1. B: ROC curves for differentiating between active and inactive in VAs patients using HMGB1. C: ROC curves for differentiating between renal involvement and without renal involvement in VAs patients using HMGB1. D: ROC curves for differentiating among the VAs subsets using HMGB1. E: ROC curves for diagnosis between patients with VAs and controls using HMGB1 and Scr. F: ROC curves for differentiating between active and inactive in VAs patients using HMGB1and Scr. HMGB1 = high-mobility group box 1, ROC = receiver operating characteristic, Scr = serum creatinine, VAs = systemic vasculitis.

to define when the disease is actually in remission and most patients develop new angiographic lesions over time usually without clear manifestations of disease activity. HMGB1 is a 30 kDa non-histone, chromatin-binding protein ubiquitously expressed in eukaryotic cells.[26] HMGB1 is mainly located in the nucleus under physiological conditions where it acts as a structural component in complex with chromatin and certain cotranscriptional factors and takes on proinflammatory properties when released extracellularly.[27] HMGBl is a newly discovered late inflammatory mediator and participates in the pathogenesis of many diseases. Recent studies showed that serum HMGB1 levels are increased in several systemic disorders including sepsis, cancer, atherosclerosis, certain chronic inflammatory, and autoimmune diseases. Findings reported by Abdulahad et al showed that levels of HMGB1 in the sera of SLE patients are increased, in particular in those with active renal disease.[11] Urbonaviciute et al suggested that HMGB1 could be a valuable biomarker for SLE disease activity as its probable involvement in the pathogenesis.[28] Until now, serum HMGB1 for diagnosis and assessment of disease in patients with VAs is still unclear. In particularly, the relation between serum HMGB1 levels and PAN has not been evaluated.

In this study, we observed that serum HMGB1 levels in VAs patients were significantly higher than EH and HC, suggesting

Table 4

Estimated value of serum high mobility group box 1 levels based on the cohort.

Estimated value	VAs	Active	Renal involvement	Subsets
Sensitivity (%)	80.4 (66.9–90.2)	77.1 (59.9–89.6)	83.9 (66.3–94.5)	70.8 (48.9–87.4)
Specificity (%)	71.7 (61.4–80.6)	62.5 (35.4–84.8)	65.0 (40.8–84.6)	77.9 (57.7–91.4)
PPV (%)	61.2 (48.5–72.9)	81.8 (64.5–93.0)	78.8 (61.1–91.0)	73.9 (51.6–85.1)
NPV (%)	86.8 (77.1–93.5)	55.6 (30.8–78.5)	55.6 (30.8–78.5)	75.0 (55.1–89.3)
PLR	2.84 (2.0–4.0)	2.06 (1.1–4.0)	2.40 (1.3–4.4)	3.19 (1.5–6.8)
NLR	0.27 (0.2–0.5)	0.37 (0.2–0.7)	0.25 (0.1–0.6)	0.38 (0.2–0.7)
AUC	0.81 (0.74–0.87)	0.74 (0.60–0.85)	0.77 (0.63–0.87)	0.74 (0.60–0.86)
YI	0.521	0.396	0.489	0.486

Values in parentheses are 95% CI.

AUC = area under curve, CI = confidence intervals, NLR = negative likelihood ratio, NPV = negative predictive value, PLR = positive likelihood ratio, PPV = positive predictive value, VAs = systemic vasculitis, YI = Youden index.

that HMGB1 may be involved in the pathogenesis of VAs. Serum HMGB1 levels were significantly higher in VAs patients with active stage than in those with an inactive stage. Further analysis showed that serum HMGB1 levels were positive correlated with BVAS and Hs-CRP. These results indicated that serum HMGB1 levels could reflect the disease activity of VAs patients. Wang et al research suggest that plasma levels of HMGB1 in active patients were significantly higher than those in normal controls and patients in remission.[15] Ma et al study showed that urinary levels of HMGB1 may be associated with the disease activity in AAV patients.[29] This is similar to our results. However, de Souza et al found that HMGB1 levels were similar between AAV patients and HC and no significant differences were found regarding mean HMGB1 levels among AAV disease subsets.[17] In another study by de Souza et al found that serum HMGB1 levels did not differ between VAs patients and HC and no difference was found between VAs patients with active disease and in remission.[18] The causes of the difference between the 2 results were analyzed, and the differences between the study may be case selection and the detection methods.

Wang et al suggested that HMGB1 contributes to glomerular endothelial cell injury in VAs through enhancing endothelium–neutrophil interactions.[30] This suggests that HMGB1 may play an important role of pathogenesis in renal injury with VAs. Therefore, we further evaluated whether HMGB1 is associated with renal involvement of VAs patients. Our results showed that serum HMGB1 levels were significantly increased in VAs patients with renal involvement, compared to non-renal involvement patients. Correlation analysis showed that serum HMGB1 levels were significant positive correlated with the Scr and 24-hour proteinuria. Wang et al research suggest that circulating HMGB1 levels might reflect the renal involvement of AAV.[15] Bruchfeld et al results showed that HMGB1 is significantly increased in AAV with renal involvement.[31] It may be speculated that HMGB1 could directly be involved in the process of renal damage and reflect renal involvement of VAs patients.

In addition, multivariate logistic regression analysis showed that HMGB1 and Scr were all independent predictor associated with VAs. Furthermore, to enhance the accuracy and the efficiency of diagnosis, HMGB1 and Scr were applied as a combined biomarker were applied to plotting the ROC curve using binary logistic regression, which showed very good sensitivity or specificity compared to HMGB1 alone. We believe that the combination achieved high predictive values for assessing active VAs patients.

HMGB1 is an important proinflammatory mediator and binds to cell surface receptors to induce inflammatory responses. Among various HMGB1 receptors, RAGE, TLR2, TLR4, and TLR9 are more involved in inflammation.[32] Neutrophils, macrophages, and monocytes stimulated by inflammation could release HMGB1, which acts as an inducer of macrophage activation including the production of tumor necrosis factor-α, interleukin-1, and other proinflammatory mediators; thus, in turn, regulates cytokine expression and promotes inflammatory cell recruitment. Recent studies identified neutrophil extracellular traps is critical in the pathogenesis of AAV.[33] HMGB1 can primes neutrophils by increasing translocation of ANCA antigens to the cell surface and induction NETs formation by interacts with TLR2, TLR4, and RAGE.[34] Therefore, HMGB1 may plays an important role in AAV. TA is a large vessel VAs characterized by granulomatous inflammation of the vessel wall and the etiopathogenesis is unknown.[35] TA often has a protracted clinical course, and relapses are common.[36] Therefore, the assessment of disease is a challenge. HMGB1 can translocate outside the cell in response to injuries by being actively secreted from activated immune cells or passively released from necrosis cells. Henes et al reported that HMGB1 serum levels are significantly higher in GPA with predominant granulomatous manifestations.[14] For this reason, we evaluated associations between TA and serum HMGB1 levels. In addition, PAN is a systemic necrotizing VAs with predominant medium-sized vessel involvement.[37] The main diagnosis depends on clinical presentation, angiography, and tissue biopsy. At present, there have been few studies of biomarkers in PAN. Serum HMGB1 levels have not been evaluated in patients with PAN. Therefore, we further analyzed the subsets of VAs.

We found that serum HMGB1 levels were significantly higher in AAV patients compared with HC. This is consistent with some studies.[14,15] Besides, our result showed that serum HMGB1 levels in patients with TA were significantly higher than in HC. This is very important for the relationship between TA and HMGB1. Cell injury and necrosis caused by the inflammatory response of granulomatous tissue may be the main cause of elevated serum HMGB1. However, de Souza et al reported that patients with TA present similar serum HMGB1 levels compared with HC.[18] This could be related to the standard of the selected case group and sample size. Therefore, it needs further confirmation such as by expanding the sample size. More interestingly, we found that serum HMGB1 was significantly higher in patients with PAN than in AAV and TA patients in our study. It possibly indicating that HMGB1 is a helpful biomarker for distinguishing VAs subsets. This is new information for PAN, and may supply some help to diagnosis and assessment of PAN. In addition, in patients with PAN, there was a positive correlation between serum HMGB1 levels and Hs-CRP, Scr, and 24-hour proteinuria. However, there was no correlation between serum HMGB1 levels and BVAS in patients with PAN. These findings probably suggests that HMGB1 play a role in the pathogenesis of PAN, in particular in patients with renal involvement, and whereas its role needs further to be investigated.

5. Limitations

However, there are some limitations should be considered in this study. First, the sample size is relatively small, and will decrease the power of statistical analysis. Second, it is a cross-sectional study and we did not observe the dynamic changes of the serum HMGB1 levels, it is difficult to establish a causal relationship between HMGB1 and VAs. Third, our results are from a single center, making chance and selection bias plausible explanations for our results.

5.1. Future directions

In addition, further well-designed studies are warranted in the future. First, expanding the sample size to confirm the current findings. Second, longitudinal studies to investigate whether HMGB1 can provide prognostic information of VAs. Third, validation is required to apply the results of this study to other populations. Finally, further studies are needed to investigate whether HMGB1 participates in the pathogenesis of VAs.

6. Conclusions

In conclusion, serum HMGB1 levels might be elevated in some patients with VAs, and may reflect the disease activity and renal

involvement, especially in PAN. HMGB1 might be as a potential biomarker is helpful for the diagnosis and assessment of VAs.

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Author contributions

Bin Zhu carried out the experiments, analyzed the data, and drafted the manuscript. Nanfang Li conceived of the study, participated in the design of the study and helped to revise the manuscript, and provided final approval of the version of the submitted manuscript. Qing Zhu collected data from medical records, participated in the design of the study, and helped to revise the manuscript. Ting Wu contributed reagents/materials/analysis tools and carried out the experiments. Mulalibieke Heizati participated in the design and direction of the study and helped to revise the manuscript. Guoliang Wang collected data from medical records and analyzed the data. Xiaoguang Yao helped with the interpretation of data and revised the manuscript. Qin Luo helped with the interpretation of results and revised the manuscript. Shasha Liu collected data from medical records. Shanshan Liu collected data from medical records.

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