*Iran J Public Health, Vol. 52, No. 11, Nov 2023, pp.2390-2401*

**Original Article**

# The Expression of High Mobility Group Box-1 (HMG1) in the Peripheral Blood and its Relation with Systemic Vasculitis Patients

*Ying Qin [1], Xin Li [1], Lidong Shi [1], Yangyang Liu [1], Zhihui Wang [2], *Yue Guan [1]*

1. *Department of Rheumatology and Immunology, The Third Affiliated Hospital of Qiqihar Medical University, Heilongjiang Province, Qiqihar 161000, China*
2. *Department of Emergency Room of Internal Medicine, The Third Affiliated Hospital of Qiqihar Medical University, Heilongjiang Province, Qiqihar 161000, China*

***Corresponding Author:** Email: guanyue1987617@qmu.edu.cn

**(Received 15 Feb 2023; accepted 11 Apr 2023)**

**Abstract**

**Background:** We aimed to explore the expression of high mobility group box-1 (HMG1) in the peripheral blood of systemic vasculitis (SV) patients.

**Methods:** The peripheral blood were collected from 35 healthy controls and 35 SV patients, and the expressions of HMGB1 and pyroptosis-related markers in the samples were detected by ELISA. They were admitted to the Department of Rheumatology and Immunology of the Third Affiliated Hospital of Qiqihar Medical University, China in 2022. The severity of diseases was graded according to the diagnosis and treatment norms of SV. The correlation between HMGB1 expression level and disease-related indicators and grades were explored through Pearson correlation analysis. The specific mechanism of HMGB1 mediating the occurrence and development of diseases through the regulation of endothelial pyroptosis was clarified.

**Results:** HMGB1 expression significantly increased in the peripheral blood of SV patients compared with healthy controls ($P<0.0001$). Pearson correlation analysis indicated that HMGB1 expression level in serum gradually increased with the aggravation in SV patients. The expression levels of ASC ($P<0.0001$), IL-1β ($P=0.004$) and IL-18 ($P<0.0001$) in peripheral blood of SV patients were significantly increased, which were significantly positively correlated with HMGB1 in peripheral blood ($P<0.0001$). Recombinant HMGB1 significantly promoted the expression of ASC, IL-1β and IL-18 in vascular endothelial cells. Recombinant HMGB1 stimulation significantly activated NLRP3 inflammasome, and the additional addition of NLRP3 inhibitor significantly inhibited HMGB1-mediated endothelial pyroptosis.

**Conclusion:** HMGB1 expression was significantly high in the peripheral blood of SV patients, which was positively correlated with the severity of diseases. HMGB1 could mediate pyroptosis through activating TLR4/NF-κB/NLRP3 signaling pathway.

**Keywords:** Systemic vasculitis; High mobility group box-1; Pyroptosis

## Introduction

Systemic vasculitis (SV) is a group of systemic autoimmune diseases characterized by vascular inflammation. The main pathological features of SV are vessel wall damage and inflammation, which causes reduced blood flow or vascular obstruction, leading to ischemia and necrosis of tissues (1). According to statistics, 40-54 cases of SV per 1 million people are reported annually. SV patients usually have prodrome symptoms, physical disorders and organ-specific manifestations. Most patients may present for non-specific signs or symptoms, or may present for emergency treatment and admit for life-threatening symptoms (2). The clinical manifestations of Primary SV are similar to those of several infectious, neoplastic and autoimmune diseases (3). Therefore, early diagnosis and new therapeutic targets are extremely important for improving the prognosis in SV patients.

As a high-mobility family protein, high mobility group box-1 (HMGB1) is a non-histone nuclear protein that functions in transcription, replication, recombination, repair, and other DNA-related activities, and is involved in various acute and chronic inflammatory processes after sterile injury or microbial invasion (4). Thus, HMGB1 can play multiple roles in the pathogenesis of inflammatory and autoimmune diseases, including systemic lupus erythematosus and rheumatoid arthritis, thereby mediating the process from inflammation to repair (5). Recent study has shown higher levels of HMGB1 in the serum of patients with Kawasaki diseases and ANCA associated SV, especially in patients with granulomatosis with polyangiitis (6). However, another study showed that patients with ANCA associated vasculitis, large artery arteritis, and giant cell arteritis did not have significant changes in HMGB1 expression level in the serum of peripheral blood compared with healthy controls (7).

Therefore, we aimed to explore HMGB1 expression in the peripheral blood of SV patients and its correlation with the severity of diseases, further explores the specific molecular mechanism involved in the progression of SV, and provides a solid theoretical basis for HMGB1 as a new target for the diagnosis and treatment of SV.

## Methods

### *Clinical Case Collection*

A total of 35 adult patients in the active phase of SV were included. They were admitted to the Department of Rheumatology and Immunology of the Third Affiliated Hospital of Qiqihar Medical University, China in 2022. Another 35 patients during the same period in the outpatient clinic underwent the health examination were grouped into the control group. All patients met the diagnostic criteria for systemic vasculitis in the diagnostic guidelines of the Chinese Society of Rheumatology (8).

Diagnostic criteria: 1) Patients with clinical manifestations of multiple system involvement: Systemic fatigue, damage to multiple systems such as the kidneys, lungs, skin, central nervous system, paranasal sinuses, gastrointestinal tract, eyes, ears, and nose; 2) Patients with positive serum ANCA test results; 3) Patients with renal biopsy showing glomerulus and segmental necrotic crescentic glomerulonephritis without immunofluorescence and immune complex deposition.

Exclusion criteria: 1) Patients with severe systemic lesions; 2) Patients with other active infections, chronic inflammation or autoimmune diseases; and 3) Patients currently participating in other clinical investigations. The patients were graded according to the criteria for diagnosis and treatment of SV, the criteria: early stage: SCr<120 μmol/L, accompanied by systemic symptoms, without life-threatening organ damage; Medium stage: SCr<500 μmol/L, with other important organ dysfunction and positive ANCA; Severe stage: SCr>500 μmol/L, with systemic symptoms and positive ANCA; Refractory SV: Scr is uncertain, with any life-threatening important organ dysfunction, and ANCA is positive or negative.

All the patients enrolled in this study have signed the informed consent and the study has been ap-

proved by the Ethics Committee of the hospital: (Q) LS [2022] No.38.

### ELISA

From the included patients, 5 ml of fasting peripheral venous blood of the forearm was collected. After standing at room temperature for 15 min, the plasma was centrifuged at 1500 r/min for 10min. The upper plasma was separated and stored in the refrigerator at -80℃ for testing. HMGB1 (RJ12463, Renjie Bio), ASC (10500-1-AP, Proteintech), IL-1β (SP10180, Wuhan Saipei Bio) and IL-18 ELISA Kit (SP10189, Wuhan Saipei Bio) were used to detect the expression levels in plasma. For each well, 0.2 ml of antigen-containing sample diluted with dilution buffer was added and incubated at 37 ℃ for 1-2 h. The coating solution was removed, the wells were washed three times for 5 min each with washing buffer (containing 0.05% Tween-20), and 0.2 ml of enzyme labeled specific antibody solution diluted in dilution buffer was added to each well and incubated at 37 ℃ for 1-2 h. The coating solution was removed, the wells were washed three times for 5 min each with washing buffer (containing 0.05% Tween-20), and 0.2 ml of enzyme substrate was added to each well. After incubation at room temperature for 30 min, 2M H2SO4 was added to each well, and OD was determined by enzymic colorimeter (OPD was 492nm).

### Cell culture

Human umbilical vein vascular endothelial cells (HUVECs, Scien Cell) were purchased and cultured with RPM 1-1640 (PM150115, Procell) with the cell culture medium containing 10% FBS. Recombinant HMGB1 (HY-P70570, MCE) was added into the cell culture medium of the experimental group, and DMSO was added into the control group, both of which were cultured to the cell density of 70-90% for reserve.

### PCR

Total RNA was extracted from cells with TRIzol reagent (Invitrogen), reverse transcribed to cDNA with the reverse transcription Kit (AT401-01, Transgen) and amplified through qPCR with PCR kit (AT401-01, Transgen). The resulting values were normalized according to the reference gene GAPDH for each parallel sample. The relative expression levels of TLR2 (F: TCCGCCTCTCGGTGTCGGAA, R: AAACGGTGGCACAGGACCCC), TLR4 (F:TCAAGCCAGGATGAGGACTGGGT, R: CAGCAATGGCCACACCGGGA) and TLR7 (F: (F: TCAAGCCAGGATGAGGACTGGGT, R: CAGGGAGAGCACTTTTAAC), R: CAGCAATGGCCACACCGGGA) compared to β-actin (F: GGCGGCACCACCATGTACCC, R: GACGATGGAGGGGCCCGACT) were calculated. All the experiments were performed in triplicate.

### Western blott

Cell proteins were extracted from RIPA cell lysate (R0020, Solarbio) and PMSF protein inhibitor (ST505, Beyotime). Protein loading buffer was added after protein quantification, and the mixture was boiled for 10 min. The protein samples were subjected to SDS-PAGE gel electrophoresis and incubated with primary antibody and antibodies of ASC (ab111852, Abcam), caspase-1 (ab207802, Abcam) and GSDMD (ab219800, Abcam). Finally, the developing liquid drops were added to PVDF membrane. Imaging scanner (eBLOT XLi) was adopted to scan the film and ImageJ 1.8.0 software to analyze the expression levels of ASC, caspase-1 and GSDMD under HMGB1 stimulation.

### Flow cytometry

The cells of different groups after treatment were collected and prepared as cell suspension containing 1-2 * 10^6 / EP tube, centrifuged at 3500 rpm, at 4℃ for 5 min, fixed with 95% ethanol for 4℃, which was then incubated with PI dye (ST511, Beyotime) and caspase-1 antibody (ab207802, Abcam) in dark for 30 min, washed with 1 ml of PBS, re-suspended again with 200 ul of PBS and analyzed for apoptosis levels through flow cytometry (BD Biosciences).

### *ChIP*

When the cell density increased to 80-90%, formaldehyde with a final concentration of 1% was added and incubated at 37℃ for 10 min. Glycinate with a final concentration of 0.125 M was added and placed at room temperature for 5 min to terminate the crosslinking. The cells were washed twice with cold PBS. Cells were collected with a cell scraper into a 15-ml centrifuge tube, centrifuged at 2000 rpm for 5 min after precooling, and the supernatant was discarded. After adding SDS lysate and protease inhibitor complex, ultrasonic crushing was carried out, and centrifuged at 10000g 4℃ for 10 min to remove insoluble substances. And 300 ul of lysate was kept, which was then divided into control group and experimental group. The operation was carried out according to ChIP kit (17-295, Sigma-aldrich), and RNA was extracted from the final precipitate for PCR.

### *Statistical analysis*

The data results were analyzed with SPSS 23.0 software (IBM Corp., Armonk, NY, USA). Measurement data such as the expression levels of HMGB1 and pyroptosis markers in peripheral serum of healthy controls and SV patients were expressed as mean±standard deviation ($\bar{x}\pm s$). The comparison of expression levels between the two groups was performed with the independent samples t-test. Whether there were differences in HMGB1 expression level between patients with different degrees of diseases and the results in cells of different groups were analyzed through one-way ANOVA combined with Tukey test. Spearman correlation analysis was used to test the correlation between HMGB1 and pyroptosis markers. $P<0.05$ was taken as statistically significant.

## Results

### *HMGB1 expression significantly increased in the peripheral blood of SV patients and was positively correlated with the severity of diseases*

The main clinical characteristics data of the patients are detailed in Table 1. The results showed that HMGB1 expression significantly increased in the peripheral blood of SV patients compared with healthy controls (8.55±0.19 vs 32.93±3.04 ng/ml, $P<0.0001$) (Fig. 1A), further Pearson correlation analysis indicated that HMGB1 expression level in serum gradually increased with the aggravation of SV patients, and HMGB1 expression level in severe and refractory patients was significantly higher than that in mild and moderate patients (Fig. 1B), indicating that HMGB1 expression level may be correlated with the severity of diseases in SV patients.

**Table 1:** General conditions of included patients

| *Variable* | *Control Group (n=35)* | *SV patients (n=35)* | *P* |
|---|---|---|---|
| Gender (Male/Female) | 16/19 | 14/21 | 0.629 |
| Age (Years old) | 41.91±1.28 | 42.20±0.92 | 0.857 |
| Disease Grading | | | |
| Mild | | 3 | |
| Moderate | | 15 | |
| Severe | | 14 | |
| Refractort | | 3 | |

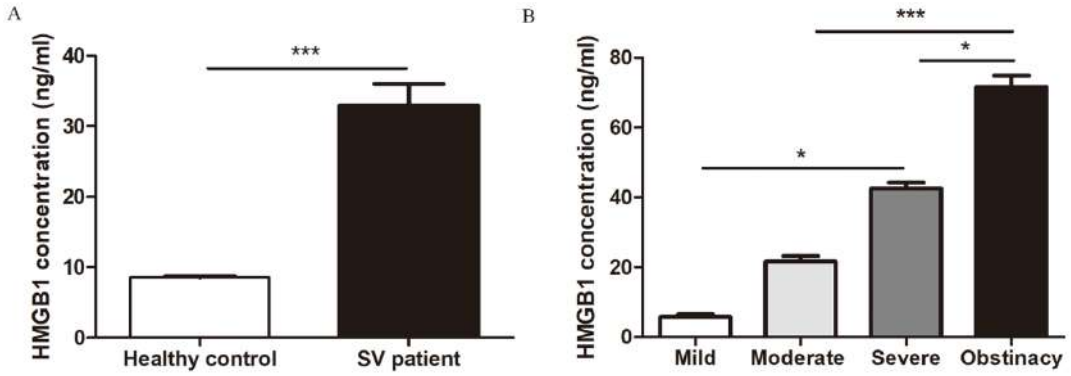


**Fig. 1:** HMGB1 expression significantly increased in the peripheral blood of SV patients and was positively correlated with the severity of diseases

Note: A: HMGB1 expression in the peripheral blood of healthy controls and SV patients; B: HMGB1 expression in the peripheral blood of patients with different grades of SV. *P*<0.05 is indicated as * and *P*<0.001 is indicated as ***

### *Expression of pyroptosis-related markers significantly increased in the peripheral blood of SV patients and was positively correlated with HMGB1 expression*

The expression levels of pyroptosis markers, ASC (4.45±0.72 vs 12.74±0.73 ng/ml, *P*<0.0001), IL-1β (0.065±0.012 vs 0.182±0.042 ng/ml, *P*=0.004) and IL-18 (0.045±0.007 vs 0.244±0.024 ng/ml, *P*<0.0001) increased significantly in SV patients by ELISA (Fig. 2A-2C), and all expression levels of ASC ($r^2$=0.4876, *P*<0.0001), IL-1β ($r^2$=0.2354, *P*=0.0031) and IL-18 ($r^2$=0.424, *P*<0.0001) showed a significant positive correlation with HMGB1 expression levels in the peripheral blood (Fig. 2D-F).

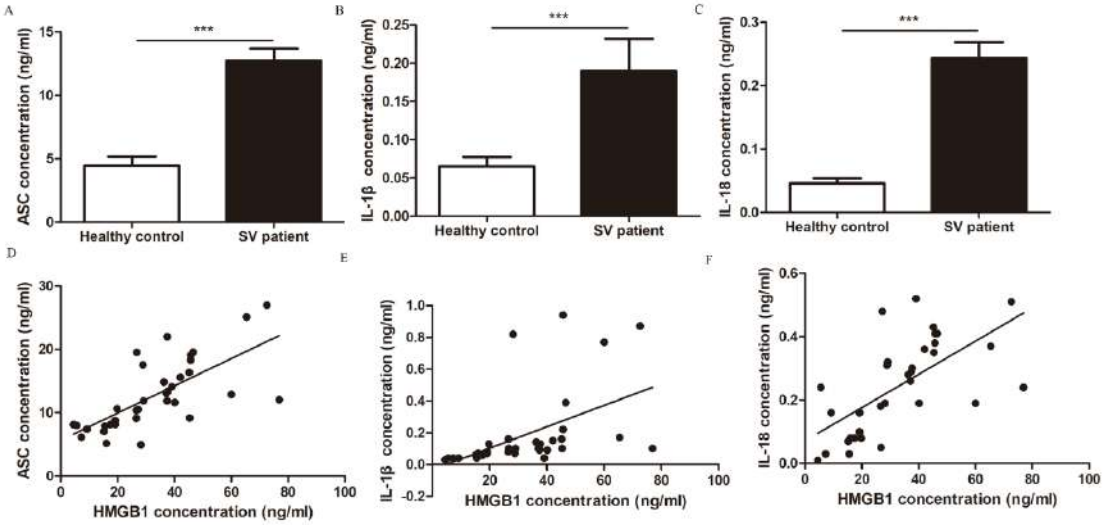


**Fig. 2:** Expression of pyroptosis-related markers in the peripheral blood of SV patients significantly increased and was positively correlated with HMGB1 expression

Note: A-C: Expression levels of ASC, IL-1β and IL-18 in the peripheral blood of healthy controls and SV patients determined by ELISA; D-F: Correlation between the expression levels of ASC, IL-1β and IL-18 and HMGB1 expression explored through Pearson correlation analysis respectively. *P* <0.001 is indicated as ***

### *HMGB 1 can promote vascular endothelial pyroptosis*

ELISA found that 10 mmol/L recombinant HMGB1 significantly increased the expression of ASC, IL-1β and IL-18 in the culture media of vascular endothelial cells (Fig. 3A), and WB also confirmed that the expression of caspase-1 and GSDMD in endothelial cells increased significantly after HMGB1 stimulation (Fig.3B-3C). Meanwhile, flow cytometry also suggested that HMGB1 stimulation could increase apoptotic cells (Fig.3D-3E), which all indicate that HMGB1 stimulation can promote the vascular endothelial pyroptosis.

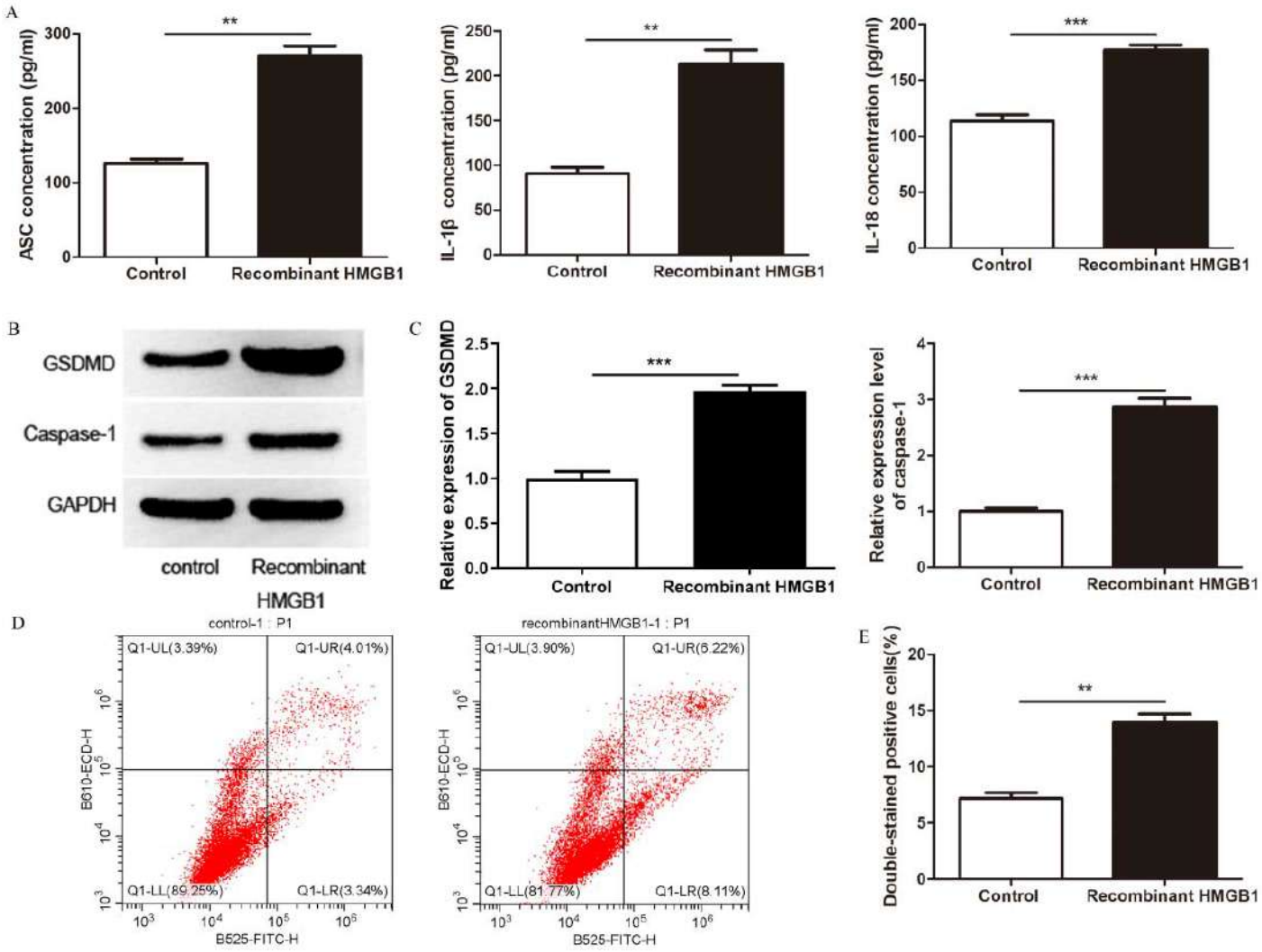


**Fig. 3:** HMGB1 can promote the vascular endothelial pyroptosis

Note: A: Expression levels of ASC, IL-1β and IL-18 in the cell culture medium of HUVECs determined by ELISA in the control group (DMSO) and the recombinant HMGB1 (10mmol/L) stimulated group; B: Expression of pyroptosis-related markers (caspase-1 and GSDMD) in HUVECs in the control group (DMSO) and the recombinant HMGB1 (10mmol/L) stimulated group detected by WB; C: WB protein result statistical diagram; D: Apoptosis of HUVECs in the control group (DMSO) and the recombinant HMGB1 (10mmol/L) stimulated group determined by flow cytometry; E: Flow cytometry statistics plot. $P<0.05$ is indicated as *, $P<0.01$ is indicated as ** and $P<0.001$ is indicated as ***

### *HMGB1 promotes the vascular endothelial pyroptosis depending on NLRP3 inflammasome activation*

HMGB1 mainly mediates NLRP3 inflammasome activation (Fig. 4A-4B). WB (Fig. 4C-4D) suggested that INF39 significantly inhibited the upregulation of HMGB1-mediated proteins, caspase-1 and GSDMD, and flow cytometry (Fig. 4E) also suggested that INF39 significantly reduced HMGB1-mediated pyroptosis cells, indicating that HMGB1 promotes vascular endothelial pyroptosis mainly depending on NLRP3 inflammasome activation.

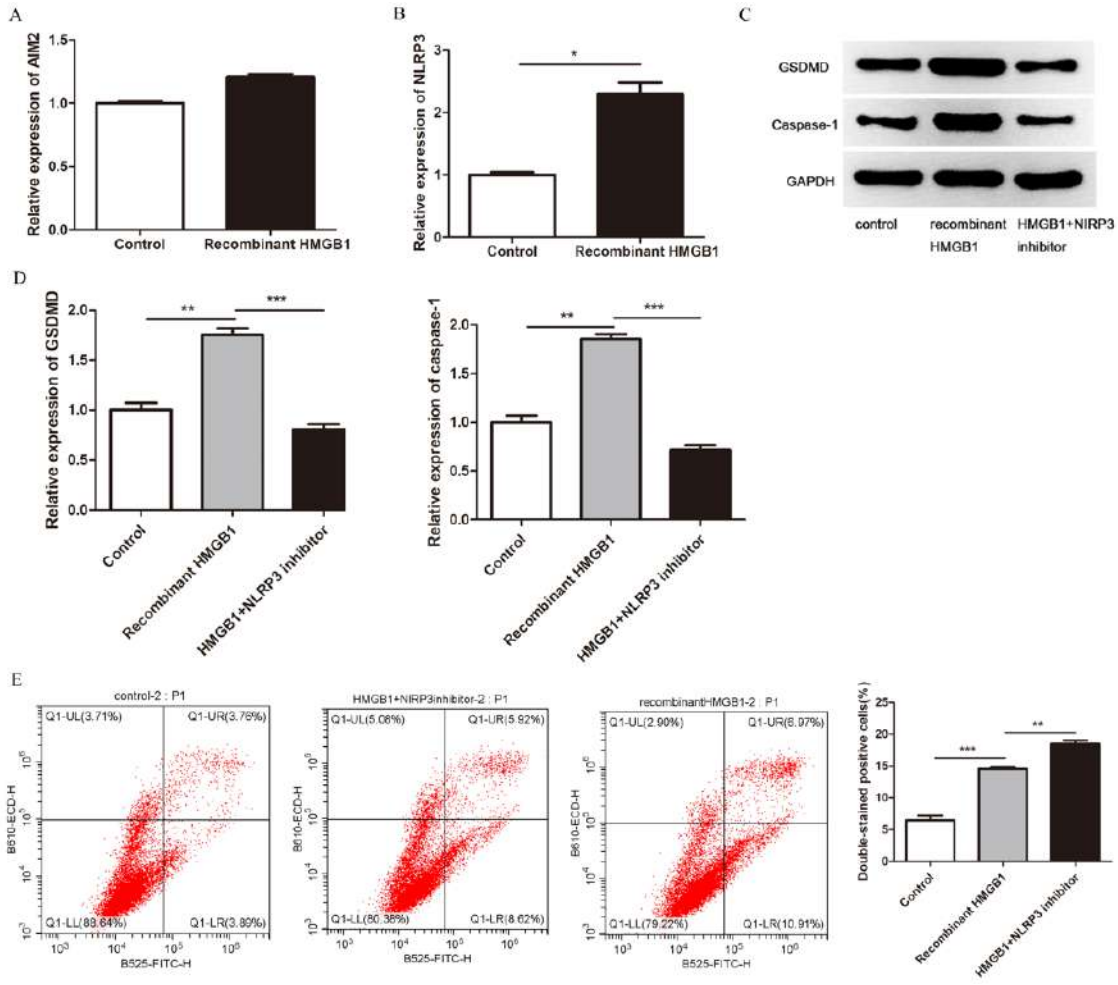


**Fig. 4:** HMGB1 promotes vascular endothelial pyroptosis depending on NLRP3 inflammasome activation
Note: A: Expression level of AIM2 in HUVECs in the control group (DMSO) and the recombinant HMGB1 (10mmol/L) stimulated group detected by PCR; B: Expression level of NLRP3 in HUVECs in the control group (DMSO) and the recombinant HMGB1 (10mmol/L) stimulated group detected by PCR; C: The control group and the recombinant HMGB stimulation group tested by WB; D: WB protein result statistical diagram; E: Apoptosis in cells of different treatment groups detected by flow cytometry. $P<0.05$ is indicated as *, $P<0.01$ is indicated as ** and $P<0.001$ is indicated as ***

### *HMGB1 activates NLRP3 inflammasome via TLR4*

PCR (Fig. 5A) and WB (Fig. 5B-5C) results suggested that recombinant HMGB1 stimulation significantly increased TLR4 expression in endothelial cells, while the expression of TLR2 and TLR7 was not significantly changed. After further addition of TLR4 inhibitor CAY10614 to the cell culture medium, WB suggested that CAY10614 could significantly reverse the promoting effect of HMGB1 on the protein expression of NLRP3, caspase-1 and GSDMD (Fig. 5D-5E), indicating that HMGB1 activates NLRP3 inflammasome via TLR4.

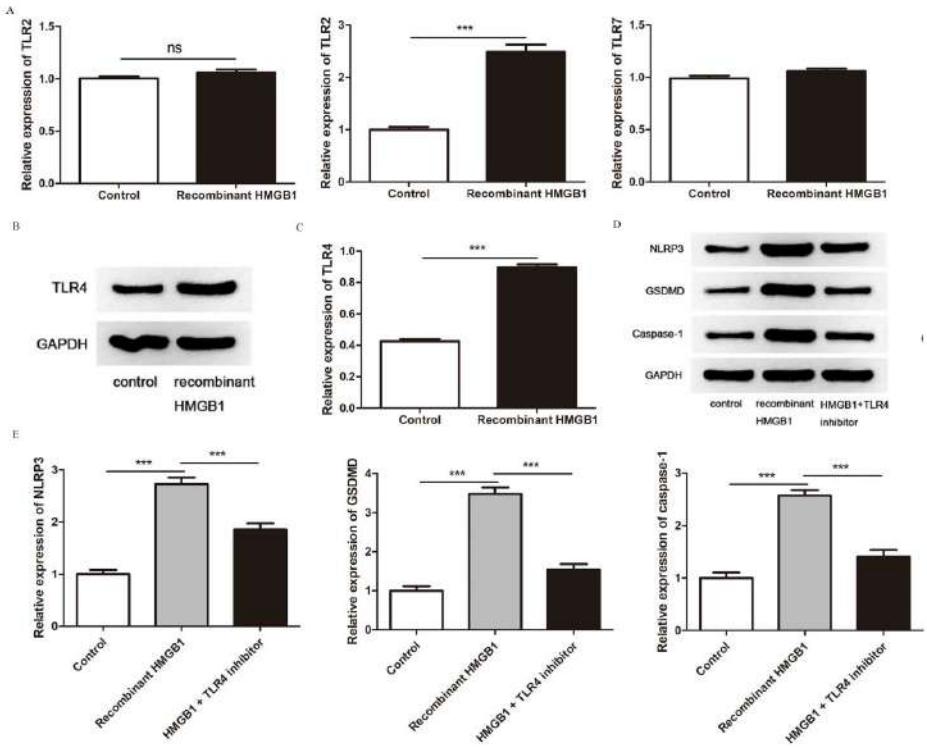


**Fig. 5:** HMGB1 promoted the vascular endothelial pyroptosis depending on NLRP3 inflammasome activation
Note: A: Expression levels of TLR2, TLR4 and TLR7 in HUVECs in the control group and the recombinant HMGB1 stimulated group detected by PCR; B: TLR4 in HUVECs in the control group and the recombinant HMGB1 stimulated group detected by WB; C: Statistical diagram of WB protein result. D: Expression levels of NLRP3, caspase-1 and GSDMD in HUVECs in the control group, the recombinant HMGB stimulation group and the recombinant HMGB1+TLR4/NF-κB inhibitor group detected by WB; E: WB protein result statistical diagram. $P<0.01$ is indicated as ** and $P<0.001$ is indicated as ***

### *HMGB 1 activates NLRP3 inflammasome through the activation of NF-κB signaling pathway via TLR4*

The up-regulation of p65 expression and inhibition of IκBα expression in NF-κB signaling pathway stimulated by recombinant HMGB1 were determined by PCR (Fig. 6A) and WB (Fig. 6B-C). ChIP (Fig. 6D) also suggested that p65 could significantly promote the protein expression of NLRP3, and the addition of TLR4 inhibitor could significantly inhibit this phenomenon, indicating that HMGB1 activates NLRP3 inflammasome by activating NF-κB signaling pathway via TLR4, the mechanism diagram is shown in Fig. 7.

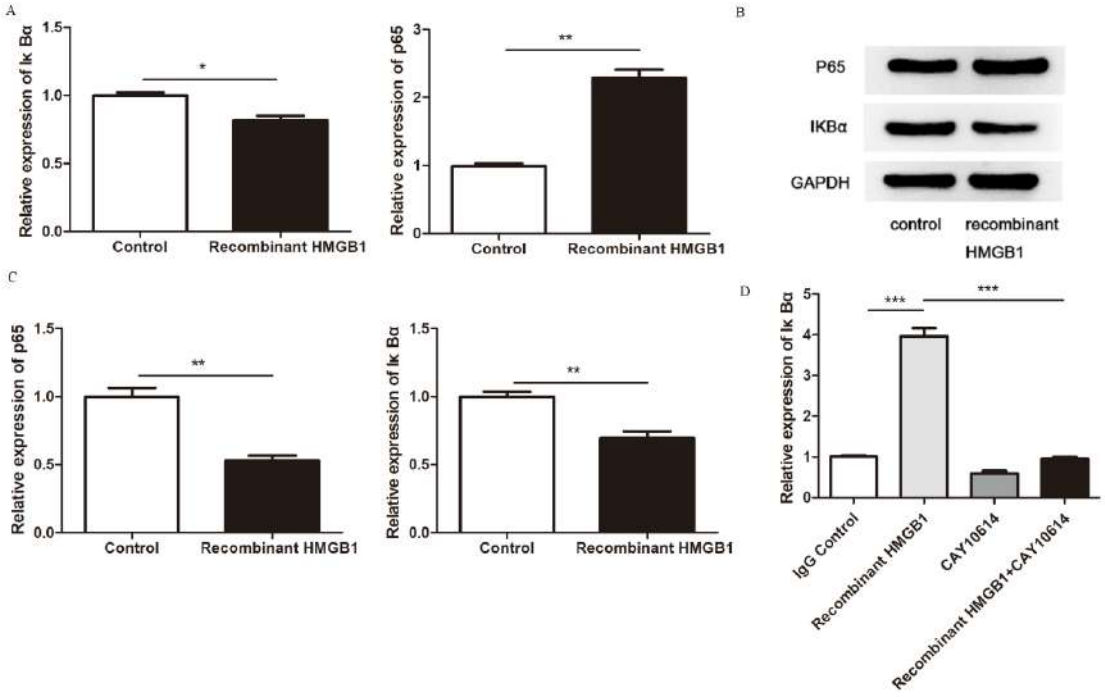


**Fig. 6:** HMGB1 activates NLRP3 inflammasome by activating NF-κB signaling pathway via TLR4
Note: A: Effect of p65 and IκBα expression levels in NF-κB signaling pathway in the control group and the recombinant HMGB1 stimulation group verified by PCR; B: Expression levels of p65 and IκBα in HUVECs of the control group and the recombinant HMGB1 stimulated group detected by WB; C: Statistical diagram of WB protein result; D: Effects of different treatment groups on NLRP3 transcription regulation investigated by chromatin immunoprecipitation experiment. $P<0.05$ is indicated as *, $P<0.01$ is indicated as ** and $P<0.001$ is indicated as ***

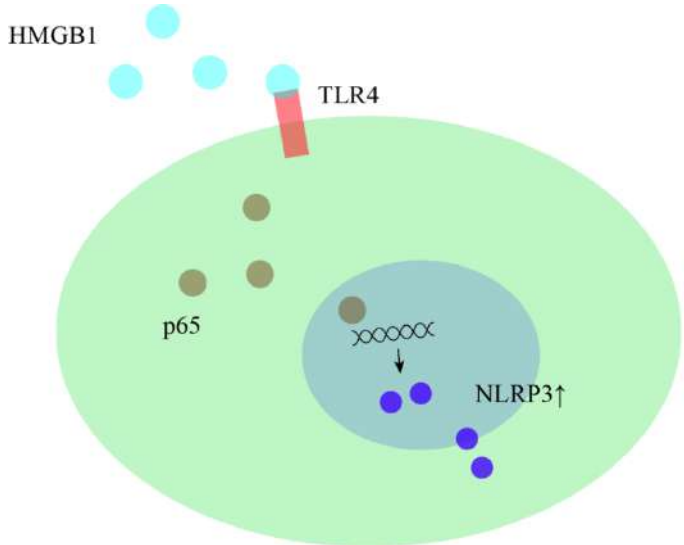


**Fig. 7:** Schematic diagram of HMGB1 activating NLRP3 inflammasome through TLR4/NF-κB signaling pathway, thus participating in the pathogenesis of SV

## Discussion

SV causes reduced blood flow or vascular obstruction, leading to ischemia and necrosis of tissues (9). At present, domestic and foreign studies mainly hold that the mechanisms that mediate SV to cause vascular injury are immune complex deposition, humoral immune-mediated ANCA response, and cellular immune-mediated T lymphocyte activation with granuloma formation. These different pathways ultimately lead to the same pathological process of the activation of endothelial cells, vascular obstruction and dependent ischemia of tissues, which may lead to bleeding in surrounding tissues. In some cases, this can lead to a weakening of blood vessel walls, which can lead to the formation of aneurysms. For different types of SV, the factors triggering and driving their inflammatory response are unknown. Previous study evaluated the application of ESR and CRP in the diagnosis of arteritis, and found that indicators, such as ESR and CRP, had limitations as biomarkers and predictors of the activity or outbreak of diseases (10).

HMGB1 belongs to the HMG-1/-2 sub-family and is a highly conserved single-peptide chain consisting of 215 amino acid residues. HMGB1 is usually actively secreted by immune cells or released by injured or dying cells, mediating the inflammatory response through the binding of various cell surface receptors (11). Within the nucleus, HMGB1 interacts with DNA and histones to determine chromatin structure and is involved in key processes such as regulating transcription. HMGB1 was found to readily bind to other pro-inflammatory molecules (12). Extracellular, HMGB1 acts as a damage-associated molecular pattern (DAMP) that stimulates both the innate and adaptive immune systems. As an inflammatory factor, HMGB1 has been demonstrated to play an important role in autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis, as well as in acute inflammatory events, such as sepsis and trauma (13). In SLE, binding of HMGB1/nucleosome complex to TLR2 activates dendritic cells and other immune cells to produce specific anti-dsDNA and anti-histone antibodies, while nucleosomes themselves fail to initiate specific antibody responses. The mechanism of HMGB1 in the occurrence and development of SV has not been elucidated. In this study, ELISA confirmed that HMGB1 was significantly highly expressed in the peripheral blood of SV patients, and its expression level was positively correlated with the severity of diseases, which may become a marker for the diagnosis and prognosis of SV.

Previous study has found that HMGB1 is closely related to the pyroptosis of vascular cells (14). Through subablative hypertherapy of HUVECs, HMGB1 induced the pyroptosis of HUVECs, while the addition of HMGB1 inhibitor ethyl pyruvate significantly reduced the pyroptosis of HUVECs (15). In this study, it was also found that pyroptosis markers significantly increased in the peripheral blood of SV patients, which were positively correlated with HMGB1 expression, indicating whether HMGB1 can mediate the occurrence and development of diseases through the activation of pyroptosis. Previous study on acute lung injury has found that HMGB1 can induce macrophages to undergo M1-type polarization by activating AIM2 inflammasome and TLC, TLR2, TLR4 and RAGE signaling in macrophages, thus mediating the occurrence and development of inflammation (16). However, the more accepted theory is that inflammasome activation occurs mainly by mediating pyroptosis, leading to the expression of caspase-1 and its downstream effects, including the cleavage of cytokines, IL-1β and IL-18. In this study, after exploring the expression of inflammasome-related proteins, AIM2 and NLRP3, in HUVECs stimulated by recombinant HMGB1, HMGB1 promoted pyroptosis through the activation of NLRP3 and its mediated inflammasome activation, which is consistent with a previous study in acute glaucoma.

As a DAMP, HMGB1 mediates biological behavior mainly by binding to various cell surface receptors, including TLR4, TLR2 and receptor advanced glycation ends (RAGEs) (17). Recent study found that lung injury, inflammatory infil-

tration and pulmonary edema were reduced in lung injury with TNF-α, IL-1β, TLR4, MyD 88 and NF-kB (18), indicating that the ligand-receptor complex formed by HMGB1 and TLR4 can participate in the occurrence and development of diseases characterized by cell death and cell injury. NLRP3 controls the production of proinflammatory cytokines and participates in the pathogenesis of more common inflammatory diseases. NLRP3 mainly functions as an important component of inflammasome, to convert pro-IL-1β into its active form together with the adaptor molecules ASC and caspase-1. In recent years, study has shown that NLRP3 gene mutations can cause chronic autoinflammatory syndromes (19) In this study, the added HMGB1 significantly activated NLRP3 and its mediated inflammasome, and HMGB1 further mediated pyroptosis through activating NLRP3 inflammasome. TLR4 mainly mediates cellular inflammatory activation by regulating the transcription factor NF-kB signaling pathway, in which p50 and p65 exist in the form of homogeneous or heterogeneous dimers in IκBα. Stimulated by cytokines, free radicals, stress, UV, LPS and viral antigens, IκBα kinase is involved in the phosphorylation of NF-kB P65 and the degradation of ubiquitin-mediated products through the proteasome pathway. Activated NF-kB enters the nucleus and induces the expression of numerous genes associated with innate and adaptive immune regulation, cell adhesion, inflammatory response and anti-apoptotic mechanisms. Thus, as shown in the mechanistic diagram of Fig. 7, the present study clarified the transcriptional activation effect of p65 on NLRP3 through the chromatin immunoprecipitation experiment, and its activation effect on NLRP3 inflammasome was dependent on TLR4.

In this study, it was found that HMGB1 was significantly higher in the peripheral blood of SV patients than normal controls, and its expression level was positively correlated with the degree of diseases, indicating that HMGB1 may have an important role in the occurrence and development of SV. This study still has the limitations of small clinical samples and lack of in vivo animal experiments; however, some studies have initially investigated the effects in other animal models of diseases, such as systemic lupus erythematosus (13), rheumatoid arthritis (20), and organ transplantation (21). The results showed that enhancing the antagonistic effect of A box on the inflammatory function of B box through the molecular structure of HMGB1 can significantly reduce the proinflammatory response produced by macrophage activation and secretion of cytokines (22). In this study, significantly high expression of pyroptosis-related markers was also found in SV, which was closely associated with HMGB1 expression. The innovative point of this study is to further explore the specific mechanism of HMGB1 promoting the pyroptosis in endothelial cells in vitro, and the results showed that in endothelial cells, HMGB1 activates TLR4/NF-κB signaling pathway to mediate pyroptosis, while both INF39, a TLR4 inhibitor, and CAY10614, a NLRP3 inhibitor, can significantly inhibit HMGB1-mediated pyroptosis, to first reveal the important role of pyroptosis mediated by HMGB1/TLR4/NF-κB/NLRP3 signaling pathway in the pathological process of SV, providing new target mechanisms for the diagnosis, treatment and prognosis judgment markers of SV.

## Journalism Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

## Acknowledgements

This study was funded by Medical and health research project of the Health Commission in Heilongjiang Province in 2022 (20220303100648).

## Conflict of Interest

The authors declare that there is no conflict of interest.

## References


1. Dharma P, Sharma S, Baltaro R, et al (2011). Systemic vasculitis. *Am Fam Physician*, 83(5): 556-565.
2. Koening CL (2013). New concepts in the pathogenesis and treatment of vasculitis syndromes. *Curr Opin Rheumatol*, 25(1): 2.
3. Moiseev S, Cohen Tervaert JW, Arimura Y, et al (2020). 2020 international consensus on ANCA testing beyond systemic vasculitis. *Autoimmun Rev*, 19(9): 102618.
4. Yang H, Wang H, Andersson U (2020). Targeting Inflammation Driven by HMGB1. *Front Immunol*, 11:484.
5. Magna M, Pisetsky DS (2014). The role of HMGB1 in the pathogenesis of inflammatory and autoimmune diseases. *Mol Med*, 20(1): 138-146.
6. Qian B, Huang H, Cheng M, et al (2020). Mechanism of HMGB1-RAGE in Kawasaki disease with coronary artery injury. *Eur J Med Res*, 25(1): 8.
7. Zhu B, Li N, Zhu Q, et al (2019). Association of serum high mobility group box 1 levels with disease activity and renal involvement in patients with systemic vasculitis. *Medicine (Baltimore)*, 98(6): e14493.
8. Xu PC, Chen M, Zhao MH (2013). Antineutrophil cytoplasmic autoantibody-associated vasculitis in Chinese patients. *Clin Exp Nephrol*, 17(5): 705-707.
9. Ferro F, Quartuccio L, Monti S, et al (2021). One year in review 2021: systemic vasculitis. *Clin Exp Rheumatol*, 39 Suppl 129(2): 3-12.
10. Grayson PC, Monach PA, Pagnoux C, et al (2015). Vasculitis Clinical Research Consortium. Value of commonly measured laboratory tests as biomarkers of disease activity and predictors of relapse in eosinophilic granulomatosis with polyangiitis. *Rheumatology (Oxford)*, 54(8): 1351-1359.
11. Andersson U, Harris HE (2010). The role of HMGB1 in the pathogenesis of rheumatic disease. *Biochim Biophys Acta*, 1799 (1-2): 141-148.
12. Venereau E, Casalgrandi M, Schiraldi M, et al (2012). Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release. *J Exp Med*, 209(9): 1519–1528.
13. Liu T, Son M, Diamond B (2020). HMGB1 in Systemic Lupus Erythematosus. *Front Immunol*, 11: 1057.
14. Kuo KC, Yang YL, Lo MH, et al (2021). Increased Expression of Pyroptosis in Leukocytes of Patients with Kawasaki Disease. *Diagnostics (Basel)*, 11(11):2035.
15. Yang M, Yang X, Wang S, et al (2019). HMGB1-induced endothelial cell pyroptosis is involved in systemic inflammatory response syndrome following radiofrequency ablation of hepatic hemangiomas. *Am J Transl Res*, 11(12): 7555-7567.
16. Wang J, Li R, Peng Z, et al (2020). HMGB1 participates in LPS-induced acute lung injury by activating the AIM2 inflammasome in macrophages and inducing polarization of M1 macrophages via TLR2, TLR4, and RAGE/NF-κB signaling pathways. *Int J Mol Med*, 45(1): 61-80.
17. Wang S, Zhang Y (2020). HMGB1 in inflammation and cancer. *J Hematol Oncol*, 13(1): 116.
18. Wu Y, Wang Y, Gong S, et al (2020). Ruscogenin alleviates LPSinduced pulmonary endothelial cell apoptosis by suppressing TLR4 signaling. *Biomed Pharmacother*, 125: 109868.
19. Li Z, Guo J, Bi L (2020). Role of the NLRP3 inflammasome in autoimmune diseases. *Biomed Pharmacother*, 130: 110542.
20. Kaur I, Behl T, Bungau S, et al (2020). Exploring the therapeutic promise of targeting HMGB1 in rheumatoid arthritis. *Life Sci*, 258: 118164.
21. Braza MS, van Leent MMT, Lameijer M, et al (2018). Inhibiting Inflammation with Myeloid Cell-Specific Nanobiologics Promotes Organ Transplant Acceptance. *Immunity*, 49(5): 819-828.e6.
22. Yang H, Ochani M, Li J, et al (2004). Reversing established sepsis with antagonists of endogenous high-mobility group box 1. *Proc Natl Acad Sci U S A*, 101(1): 296-301.