# Clinical characteristics of macrophage activation syndrome in adult-onset Still's disease

Q. Gao[1], Y. Yuan[1], Y. Wang[2], J. Jiang[2], Z. Ye[1], T. Liu[1], Z. Jiang[1], L. Zhao[1]

[1]Department of Rheumatology, The First Hospital of Jilin University, Changchun, Jilin;
[2]Division of Clinical Research, The First Hospital of Jilin University, Changchun, Jilin, China.

Qi Gao, PhD
Yi Yuan, PhD
Yueqi Wang, MM
Jing Jiang, PhD
Zhuang Ye, PhD
Tao Liu, PhD
Zhenyu Jiang, PhD*
Ling Zhao, PhD*

*These authors contributed equally.

Please address correspondence to:
Ling Zhao,
Department of Rheumatology,
The First Hospital of Jilin University,
1 Xinmin Street, Changchun,
Jilin 130021, China.
E-mail: zhaoling52@163.com

and
Zhenyu Jiang,
E-mail: jiangzhenyu2012@163.com

Received on February 18, 2021; accepted in revised form on May 20, 2021.

Clin Exp Rheumatol 2021; 39 (Suppl. 132): S59-S66.



**Key words:** adult-onset Still's disease, macrophage activation syndrome, survival analysis

Funding: the present study was supported by the funding from Jilin Province Direct Health Special Project (JLSWSRCZX2020-0034), Jilin Province Direct Health Special Project (JLSWSRCZX2020-0038) and the Tenth Youth Fund of The First Hospital of Jilin University (JDYY102019014).

Competing interests: none declared.

## ABSTRACT

**Objective.** *Macrophage activation syndrome (MAS) is considered the most severe complication of adult-onset Still's disease (AOSD). This retrospective observational study was conducted to explore the clinical characteristics of AOSD-MAS patients, the risk factors for MAS in AOSD and prognostic factors in AOSD. Early changes in the clinical characteristics of AOSD-MAS were also studied.*

**Methods.** *111 hospitalised AOSD patients were included in this retrospective analysis and analysed for the features of AOSD-MAS, selecting independent risk factors associated with MAS and the correlations between clinical characteristics and patient survival.*

**Results.** *Nine subjects (8.1%) developed MAS. AOSD-MAS patients had a higher incidence of jaundice (33.3% vs. 2.9%, p=0.007) and aspartate aminotransferase (AST) greater than 5-fold (33.3% vs. 2.9%, p=0.007). Jaundice was associated with an increased risk of MAS (OR=16.50, 95% CI: 2.73-99.82, p=0.002). The AOSD-MAS group had a higher mortality rate (55.6% vs. 8.0%, p=0.001). MAS (HR=11.22, 95% CI: 3.46-36.38, p<0.001), and white blood cell (WBC) greater than $20\times10^9$/L (HR=5.80, 95% CI: 1.09-30.92, p=0.040) were independent prognostic factors for death in AOSD patients. In the AOSD-MAS group, transaminase, triglycerides (TGs) and serum ferritin (SF) were elevated in the early disease stage, sometimes earlier than changes in blood cells in MAS.*

**Conclusion.** *MAS occurrence significantly reduced the survival rate of patients with AOSD. The presence of jaundice was associated with MAS occurrence. MAS and a WBC count $>20\times10^9$/L were associated with a high risk of AOSD-related death. AOSD should alert the possibility of MAS when elevated transaminase, TGs and SF cannot be explained.*

## Introduction

Still's disease is a class of exclusive autoinflammatory diseases involving multiple organs, and the complete pathogenesis is currently unknown. Still's disease is divided by age: that occurring in individuals 16 years old or younger is referred to as systemic juvenile idiopathic arthritis (sJIA), and that occurring in individuals more than 16 years old is referred as adult-onset Still's disease (AOSD) (1, 2). Clinical manifestations are fever, rash, joint pain, hepatomegaly, splenomegaly, lymphadenopathy, serositis, etc. (2). Among them, fever (3, 4), rash (5), and joint pain (3) are the most common symptoms. Laboratory tests are characterised by elevated white blood cell (WBC), transaminase changes, and elevated serum ferritin (SF) (2). Importantly, elevated SF is meaningful for diagnosis, especially SF increased more than 5-fold (6). Generally, AOSD should be considered the mild form. The AOSD course can be categorised into 3 different clinical patterns: monocyclic, polycyclic, and chronic (7). Few studies have focused on the prognostic factors of AOSD. Studies have revealed that polyarthritis and joint erosion at disease onset are predictive of chronic progression and a poor functional prognosis (7, 8). Furthermore, notable liver dysfunction, splenomegaly, a low number of platelets or neutrophils, high levels of serum ferritin, and a reduced level of fibrinogen (FBG) are risk factors for poor outcome (9). A systemic score greater than 7 showed a strong prognostic impact in categorising patients at risk of AOSD-related death (10). Patients with severe complications, such as macrophage activation syndrome (MAS), disseminated intravascular coagulation, liver failure, and severe infection, have a poor prognosis (10, 11).

AOSD is one of the autoimmune diseases that is most likely to develop

MAS which is estimated to occur in 10-15% of patients (12, 13). MAS is characterised by a cytokine storm (14), haemophagocytosis and multi-organ damage (15). Its pathogenesis involves the activation of key innate immune pathways, including IL-1, IL-6 and IL-18, leading to systemic inflammation (16). MAS is a group of clinical manifestations with high fever, lymphadenopathy, hepatosplenomegaly, decreased blood cells, abnormal liver function, elevated SF, and high triglycerides (TGs) (11, 17). MAS can increase the mortality of AOSD. Previous studies have shown that the mortality of patients with AOSD-MAS and the mortality of patients with AOSD are 52.9% and 9.5%, respectively (11). The characteristics of MAS are very similar to those of AOSD, so it is very difficult to diagnose AOSD with MAS early. Because of its non-specific clinical characteristics, MAS is not easy to distinguish from other diseases, such as systemic infection and malignancy. Moreover, the 2004-HLH criteria (18) or HScore (19) is commonly used for diagnosis, but the results are not consistent. Therefore, a few studies have conducted research on the predictive indicators of MAS. Some studies have revealed predictors of MAS, including platelets (PLTs), anaemia, SF, β2-microglobulin, hepatomegaly, lymphadenopathy, abdominal pain, splenomegaly and pericarditis (11, 20, 21). Because the number of patients with AOSD-MAS is relatively insufficient and the diagnosis is relatively difficult, there are relatively few studies on it. There is no research revealing the changes in laboratory examinations results of AOSD-MAS patients.

In this study, we analysed the clinical data of 111 AOSD patients, including 9 with MAS. We aimed to explore the clinical characteristics of AOSD-MAS patients and to identify the risk factors for death in AOSD patients. More importantly, we described the changing clinical characteristics of AOSD-MAS patients.

## Materials and methods

### *Patients*

We performed a retrospective analysis of patients in the Department of Rheumatology of the First Hospital of Jilin University, China, between January 2014 and January 2018. All patients fulfilled the Yamaguchi criteria (22), which are considered to be the most sensitive criteria for the diagnosis of AOSD, and excluded those with infectious diseases, malignancy or other autoimmune diseases. The Yamaguchi criteria are as follows: A) major criteria: 1) fever higher than 39°C lasting for more than 1 week; 2) arthralgia/arthritis lasting for more than 2 weeks; 3) typical rash and 4) WBC count greater than 10,000 with 80% neutrophils; B) minor criteria: 1) sore throat; 2) lymphadenopathy; 3) increased liver function tests and 4) rheumatoid factor (RF) and antinuclear antibody (ANA) negative. Five or more criteria are required, two or more of which must be major. We excluded patients with infections by blood cultures or bone marrow (BM) cultures, serology and PCR analyses, chest computed tomography (CT) and abdominal CT. We evaluated possible differential diagnoses with malignancies by chest CT, abdominal echography, abdominal CT and blood samples. For patients with possible haematologic cancers, we also performed a BM examination and lymph node biopsy. Autoimmune diseases were excluded by blood tests, ANA, anticitrullinated peptide antibodies, RF, and antineutrophil cytoplasmic antibodies. All the AOSD-MAS patients met the 2004-HLH criteria (18) and HScore (scores greater than 169 were 93% sensitive and 86% specific, as shown in Supplementary Table S1) (19). The 2004-HLH criteria are as follows: 1) fever greater or equal to 38.5°C; 2) splenomegaly (craniocaudal length longer than 12 cm); 3) peripheral blood cytopenia with at least 2 of the following: haemoglobin less than 9 g/dL, PLTs less than 100,000/microL; 4) absolute neutrophil count less than 1,000/microL; 5) hypertriglyceridemia (fasting TGs greater than 265 mg/dL or hypofibrinogenemia (FBG less than 150 mg/dL); 6) haemophagocytosis in the bone marrow, spleen, lymph node, or liver; 7) SF greater than 500 ng/mL; 8) elevated soluble CD25 (soluble IL-2 receptor alpha (sIL-2R)) two standard deviations above age-adjusted laboratory-specific norms and 9) NK cell activity. For a diagnosis of HLH, 5 of the 8 criteria had to be met. In our study, 111 AOSD patients were analysed for clinical features and divided into two groups: AOSD-MAS and AOSD-non-MAS. At baseline, each patient was investigated for the onset of MAS, and at follow-up, the occurrence of death was recorded. Methods for follow-up included outpatient medical record inquiry, inpatient medical record inquiry, telephone follow-up and death registration systems.

The Ethics Committee of the First Hospital of Jilin University approved the study protocol (NO. 2013-268), and it was performed according to the Declaration of Helsinki. After approval of our ethics committee, we collected written informed consent from patients who were presently and actively followed-up. The survival time was defined as the duration from the date of admission to March 1, 2018. Patients who were lost at the time of interview were excluded from the survival analysis. The overall survival rate was used to define patient prognosis. However, since this study was retrospective, for 13 dead patients and 2 patients who were lost to follow-up, we used the fully anonymised clinical data only for research purposes without any other intended aim.

### *Clinical and laboratory data*

The clinical characteristics, laboratory data, disease course, treatment, MAS and survival data of all patients were recorded at the first visit to our hospital of this current onset. Clinical data included sex, age, peak fever temperature, rash, arthritis, hepatosplenic lymphadenopathy, treatment and prognosis. Laboratory tests included routine blood tests, SF levels, liver function tests, C reactive protein (CRP), erythrocyte sedimentation rate (ESR), FBG, TGs, RF, ANA and bone marrow characteristics. The treatments were recorded as three groups: first-line therapy, including non-steroidal anti-inflammatory drugs (NSAIDs) and/or steroids; second-line therapy, including steroids and conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) and/or intravenous immunoglobulin (IVIg);

and biologic agent therapy: steroids and csDMARDs and/or IVIg and biologic agents (23). The median time from admission to the diagnosis of AOSD-MAS was 17 days. Therefore, AOSD-non-MAS patients received treatment within 17 days of admission. For AOSD-MAS patients, data on treatment before the diagnosis of MAS were collected.

Data from AOSD-MAS patients were collected at 4 time points: the baseline, before MAS, diagnosed MAS and outcome. The baseline was defined as the first visit to our hospital of this current onset. To identify the changing features of MAS, we collected the most recent data before blood cells were reduced (*i.e.*, before MAS). Diagnosed MAS data were recorded at the time of MAS diagnosis. The outcome time point was within 24 hours before discharge or death. Patient 5 died in 3 days, and some of the data were not comprehensive.

*Statistical analysis*

The categorical variables are presented as frequencies with percentages and were compared with the $\chi^2$ test or Fisher's exact test when appropriate. Any variable having a significant univariable result ($p<0.05$) was selected as a possible candidate for multivariate logistic regression analysis to employ the independent risk factor associated with the development of MAS, and odds ratios (ORs) with their 95% confidence intervals (CIs) were calculated. Survival curves within each stratification of variables were plotted with the Kaplan-Meier method and compared by the log-rank test. The multivariate Cox proportional hazards model was used to evaluate the prognostic roles of clinical and laboratory variables and hazard ratios (HRs) with their 95% CIs were calculated. Statistical analysis was performed using SPSS 20.0 statistical software (SPSS Inc.; Chicago, USA). A *p*-value less than 0.05 was defined as statistically significant.

## Results

*Clinical characteristics of the evaluated patients*

The demographic and clinical features of the 111 investigated patients are shown in Table I. In total, 111 AOSD

**Table I.** Clinical and laboratory characteristics of AOSD patients according to with or without MAS (n=111).

| Characteristics | | AOSD n (%) | AOSD-nonMAS n (%) | AOSD-MAS n (%) | *p* |
|---|---|---|---|---|---|
| Age (years) | ≤45 | 62 (55.9) | 58 (56.9) | 4 (44.4) | 0.505 |
| | >45 | 49 (44.1) | 44 (43.1) | 5 (55.6) | |
| Gender | male | 27 (24.3) | 26 (25.5) | 1 (11.1) | 0.335 |
| | female | 84 (75.7) | 76 (74.5) | 8 (88.9) | |
| Peak temperature (°C) | 37.3-38.0 | 3 (2.7) | 3 (2.9) | 0 (0.0) | 0.098 |
| | 38.1-39.0 | 38 (34.2) | 36 (35.3) | 2 (22.2) | |
| | 39.1-41.0 | 66 (59.5) | 61 (59.8) | 5 (55.6) | |
| | >41.0 | 4 (3.6) | 2 (2.0) | 2 (22.2) | |
| Rash | no | 18 (16.2) | 16 (15.7) | 2 (22.2) | 0.637 |
| | yes | 93 (83.8) | 86 (84.3) | 7 (77.8) | |
| Sore throat | no | 77 (69.4) | 70 (68.6) | 7 (77.8) | 0.440 |
| | yes | 34 (30.6) | 32 (31.4) | 2 (22.2) | |
| Jaundice | no | 105 (94.6) | 99 (97.1) | 6 (66.7) | **0.007** |
| | yes | 6 (5.4) | 3 (2.9) | 3 (33.3) | |
| Arthritis | no | 50 (45.0) | 46 (45.1) | 4 (44.4) | 1.000 |
| | yes | 61 (55.0) | 56 (54.9) | 5 (55.6) | |
| Lymphadenopathy | no | 78 (70.3) | 72 (70.6) | 6 (66.7) | 1.000 |
| | yes | 33 (29.7) | 30 (29.4) | 3 (33.3) | |
| Hepatomegaly | no | 109 (98.2) | 100 (98.0) | 9 (100) | 1.000 |
| | yes | 2 (1.8) | 2 (2.0) | 0 (0.0) | |
| Splenomegaly | no | 91 (82.0) | 85 (83.3) | 6 (66.7) | 0.204 |
| | yes | 20 (18.0) | 17 (16.7) | 3 (33.3) | |
| Anaemia | no | 37 (33.3) | 36 (35.3) | 1 (11.1) | 0.267 |
| | yes | 74 (66.7) | 66 (64.7) | 8 (88.9) | |
| SF (ug/L) | <1000 | 9 (8.1) | 9 (8.8) | 0 (0.0) | 0.653 |
| | 1000-10000 | 67 (60.4) | 61 (59.8) | 6 (66.7) | |
| | >10000 | 35 (31.5) | 32 (31.4) | 3 (33.3) | |
| PLT ($\times10^9$/L) | ≤100 | 6 (5.4) | 5 (4.9) | 1 (11.1) | 0.121 |
| | 101-400 | 90 (81.1) | 85 (83.3) | 5 55.6) | |
| | >400 | 15 (13.5) | 12 (11.8) | 3 (33.3) | |
| WBC ($\times10^9$/L) | <10 | 29 (26.1) | 26 (25.5) | 3 (33.3) | 0.423 |
| | 10-20 | 62 (55.9) | 57 (55.9) | 5 (55.6) | |
| | >20 | 20 (18.0) | 19 (18.6) | 1 (11.1) | |
| TG (mmol/L) | 0.28-1.8 | 57 (82.6) | 54 (85.7) | 3 (50.0) | 0.061 |
| | >1.8 | 12 (17.4) | 9 (14.3) | 3 (50.0) | |
| AST | ≤5 folds | 105 (94.6) | 99 (97.1) | 6 (66.7) | **0.007** |
| | 5-15 folds | 4 (3.6) | 2 (2.0) | 2 (22.2) | |
| | ≥20 folds | 2 (1.8) | 1 (0.9) | 1 (11.1) | |
| ALT | ≤5 folds | 102 (91.9) | 94 (92.2) | 8 (88.9) | 0.758 |
| | 5-15 folds | 6 (5.4) | 5 (4.9) | 1 (11.1) | |
| | ≥20 folds | 3 (2.7) | 3 (2.9) | 0 (0.0) | |
| ESR (mm/h) | 0-20 | 9 (8.1) | 7 (6.9) | 2 (22.2) | 0.156 |
| | >20 | 102 (91.9) | 95 (93.1) | 7 (77.8) | |
| CRP (mg/L) | 0-3.5 | 3 (2.7) | 3 (2.9) | 0 (0.0) | 1.000 |
| | >3.5 | 108 (97.3) | 99 (97.1) | 9 (100.0) | |
| FBG (g/L) | <2 | 4 (5.3) | 3 (4.2) | 1 (25.0) | 0.252 |
| | 2-4 | 18 (23.7) | 17 (23.6) | 1 (25.0) | |
| | >4 | 54 (71.0) | 52 (72.2) | 2 (50.0) | |
| Treatment | First-line therapy | 76 (68.5) | 72 (70.6) | 4 (44.4) | 0.060 |
| | Second-line therapy | 33 (29.7) | 29 (28.4) | 4 (44.4) | |
| | Biologic agent therapy | 2 (1.8) | 1 (1.0) | 1 (11.2) | |
| Outcome | death | 13 (11.9) | 8 (8.0) | 5 (55.6) | **0.001** |
| | alive | 96 (88.1) | 92 (92.0) | 4 (44.4) | |

Statistical significance was expressed by *p*-value <0.05.

The first-line therapy is defined as treated with non-steroidal anti-inflammatory drugs (NSAIDs) and/or steroid; the second-line therapy is defined as treated with steroid and/or conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs) and/or intravenous immunoglobulin (IVIg); the biologic agent therapy is defined as treated with steroid and/or csDMARDs and/or IVIg and/or biologic agent.

SF: serum ferritin; PLT: platelet; WBC: white blood cells; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; FBG: fibrinogen; TG: triglyceride; MAS: macrophage activation syndrome.

**Table II.** Logistic regression analyses for MAS risk in AOSD patients (n=111).

| Characteristics | | n (%) | Crude OR (95% CI) | *p* | Adjusted OR (95% CI) | *p* |
|---|---|---|---|---|---|---|
| Jaundice | no | 105 (94.6) | 1.00 | **0.002** | 1.00 | **0.002** |
| | yes | 6 (5.4) | 16.50 (2.73,99.82) | | 16.50 (2.73-99.82) | |
| AST | ≤5 folds | 105 (94.6) | 1.00 | **0.009** | - | |
| | 5-15 folds | 4 (3.6) | 16.49 (1.97,138.11) | | - | |
| | ≥20 folds | 2 (1.8) | 16.50 (0.92,297.40) | | - | |

After adjusted variables have statistical significance in univariate logistic analyses: jaundice and AST.

patients, including 84 females (75.7%), and 102 AOSD-nonMAS patients, including 26 males (25.5%) and 76 females (74.5%), were analysed in this study. One male (11.1%) and 8 females (88.9%) were comprised the 9 AOSD-MAS patients. The median ($P_{25}$, $P_{75}$) age at AOSD diagnosis was 41 (29, 35) years. All patients had fever (100%), and the common clinical manifestations were rash (83.8%), arthritis (55.0%), and sore throat (30.6%). At baseline, the common manifestations of fever, rash, and arthritis were similar between the two groups. The incidence of jaundice was higher in the AOSD-MAS group than in the AOSD-nonMAS group (33.3% *vs*. 2.9%, *p*=0.007). Inflammatory markers such as WBCs, ESR, CRP and FBG were increased in AOSD. These increases were accompanied by increases in transaminase and SF. The increased aspartate aminotransferase (AST) level in the AOSD-MAS group was mainly greater than 5-fold (compared with the upper normal limits), and that in the AOSD-nonMAS group was mainly less than or equal to 5-fold (*p*=0.007). Although the proportion of patients with elevated TGs was higher in the AOSD-MAS group than in the AOSD-nonMAS group, this difference did not reach statistical significance (50.0% *vs*. 14.3%, *p*=0.061). First-line therapy accounted for 68.5% of AOSD patients, 70.6% of AOSD-nonMAS patients, and 44.4% of AOSD-MAS patients. The application rate of second-line therapy was higher in AOSD-MAS patients (44.4% *vs*. 28.4%). Early onset of treatment was not significantly different between the

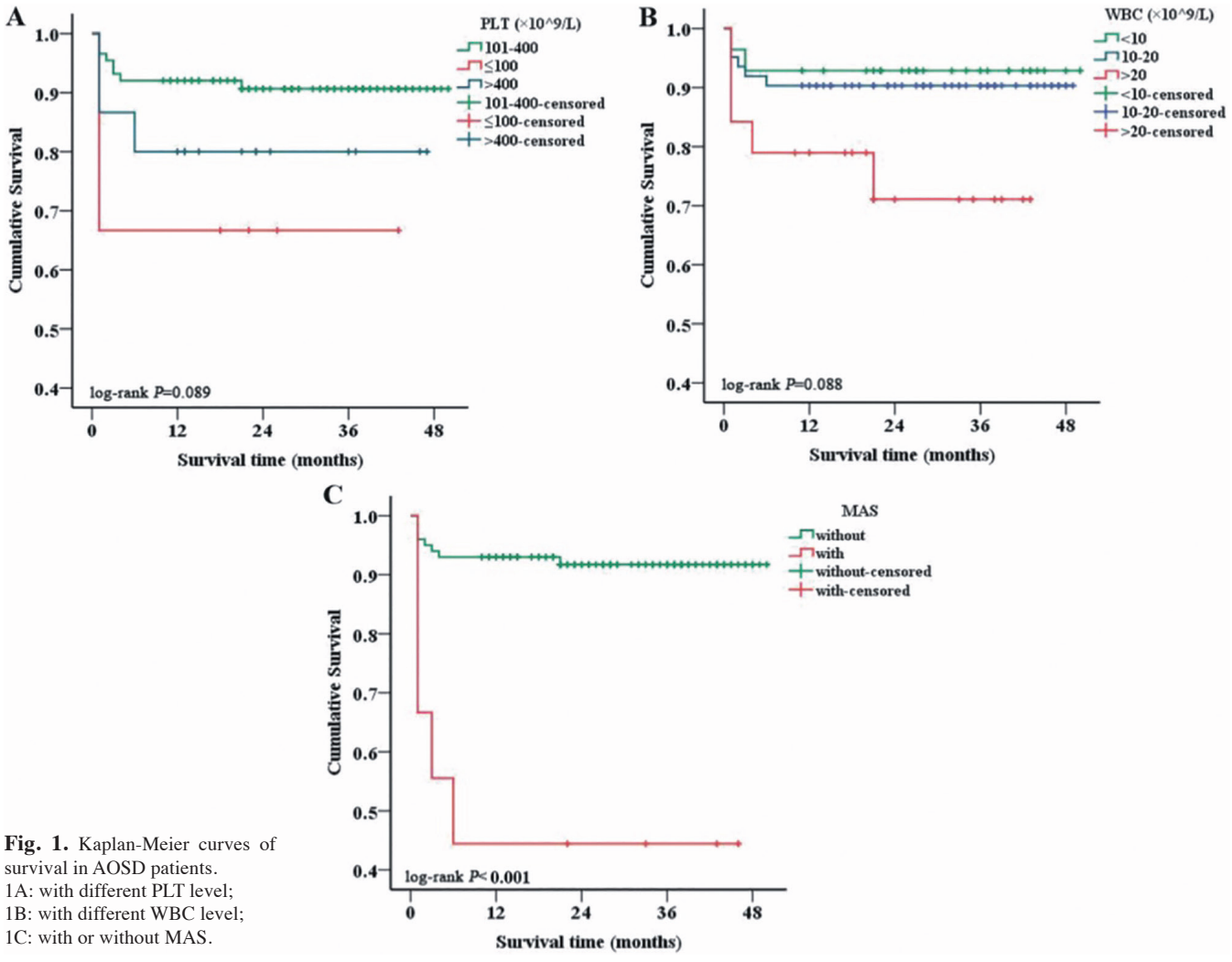


**Fig. 1.** Kaplan-Meier curves of survival in AOSD patients.
1A: with different PLT level;
1B: with different WBC level;
1C: with or without MAS.

two groups ($p$=0.060). One AOSD-non-MAS patient was given biologic agents because she had persistent fever and joint pain after using steroids and methotrexate, and she achieved remission.

The median follow-up time was 28 months, 96 (86.5%) patients survived, 13 (11.7%) patients died, and 2 patients (1.8%) were lost to follow-up. In total, 13 patients died, 5 of whom were complicated with MAS. Six AOSD-non-MAS patients died due to septic shock, and 2 died because of infection and multiple organ dysfunction syndrome (MODS). AOSD-MAS patients had a higher mortality rate (55.6% *vs*. 8.0%, $p$=0.001).

*Risk factors for MAS occurrence*

Univariate and multivariate logistic regression analyses were performed to investigate the possible risk factors for MAS occurrence, and the results are shown in Table II. In the univariate analyses, evidence of the following factors was significantly associated with MAS occurrence: jaundice ($p$=0.002) and AST ranging from 5-15-fold ($p$=0.009). Conversely, the following factors were not associated with MAS: sex, age, other recorded clinical features, SF, PLTs, WBCs, ALT, ESR CRP and different therapeutic strategies. The variables that showed significant statistical significance in the univariate analysis were included in the multivariate analysis in a forward manner, and only jaundice was an independent risk factor for MAS (OR=16.50, 95% CI: 2.73–99.82, $p$=0.002).

*Analysis of overall survival in AOSD patients*

The log-rank test and multivariate Cox regression test were used to analyse the overall survival of AOSD patients. The occurrence of MAS significantly reduced the survival rate of AOSD patients (log-rank test $p$<0.001, Fig. 1C). PLTs (log-rank $p$=0.089, Fig. 1A) and WBCs (log-rank $p$=0.088, Fig. 1B) had a tendency to reduced survival rates, although the differences were not statistically significant. Three variables ($p$<0.1) were used to describe how these factors jointly impacted survival in the multivariate Cox regression analysis. A WBC greater than $20\times10^9$/L (HR=5.80, 95% CI: 1.09-30.92, $p$=0.040) and MAS (HR=11.22, 95% CI: 3.46-36.38, $p$<0.001) were independent prognostic factors related to death (Table III).

**Table III.** Multivariate COX regression test for the factors associated with AOSD prognosis.

| Characteristics | | n | Death n (%) | Adjusted HR (95% CI) | $p$ |
|---|---|---|---|---|---|
| WBC ($\times10^9$/L) | <10 | 28 | 2 (7.1) | 1.00 | |
| | 10-20 | 62 | 6 (9.7) | 1.43 (0.29-7.08) | 0.587 |
| | >20 | 19 | 5 (26.3) | 5.80 (1.09-30.92) | **0.040** |
| MAS | without | 100 | 8 (8.0) | 1.00 | |
| | with | 9 | 5 (55.6) | 11.22 (3.46-36.38) | **<0.001** |

After adjusted variables having a significant log-rank test: PLT, WBC and MAS.

**Table IV.** General information, treatment and survival outcome of AOSD-MAS patients.

| Patient | Gender | Age | HScore | Treatment | Outcome |
|---|---|---|---|---|---|
| 1 | F | 52 | 281 | a+b | death |
| 2 | F | 55 | 207 | a | alive |
| 3 | F | 50 | 261 | a+b+c+d+e+f | alive |
| 4 | F | 23 | 253 | a+b+g | alive |
| 5 | F | 41 | 170 | a | death |
| 6 | F | 46 | 284 | a+b+g | death |
| 7 | F | 51 | 262 | a+b | death |
| 8 | M | 19 | 215 | a+b | death |
| 9 | F | 30 | 281 | a+b+g | alive |

a: steroid; b: IVIg; c: cyclophosphamide; d: Tocilizumab; e: mycophenolate mofetil; f: cyclosporine; g: etoposide; F: female; M: male.

*Changing features of AOSD-MAS*

The characteristics of the 9 AOSD-MAS patients are described in Table IV and Supplementary Table S2. Three patients had jaundice at onset, and then 2 patients developed jaundice during MAS onset. Four patients were alive, and 5 had died. Throughout the entire treatment of MAS patients, 9 patients used steroids, and 7 patients used IVIg. One patient (Patient 3) was treated with biologic agents. Because of early combination therapy, Patient 3 achieved remission. Two patients (Patient 2 and Patient 5) were treated with first-line therapy. Patient 2 had minimal organ involvement (anaemia, leukopenia, and elevated transaminases). Patient 5 died but received steroid therapy because the disease progressed rapidly, and steroids cannot be used with other drugs.

Clinical manifestations such as fever, rash, arthritis, and sore throat were improved after the treatment of AOSD. However, when patients had MAS, their temperature dramatically increased, even with antipyretic drugs. When the blood cell count began to change, transaminase, SF and TGs became higher than baseline and FBG became lower, sometimes earlier than changes in blood cells. In the event of disease improvement, the transaminase and SF of the patients decreased. However, two patients showed a downward trend in transaminase at the time of death (Fig. 2 and Supplementary Fig. S1).

## Discussion

To our knowledge, this is the first study devoted to analysing the changing features of AOSD-MAS indicators, which can provide help for early AOSD-MAS warning and avoid MAS-related AOSD mortality.

Females were more affected by AOSD than men, and the ratio was similar to that reported in the study by Zeng *et al*., with 73.8% of patients being female (24). The median age at baseline was 41 years, whereas that in a previous study in Shanghai China was 37 years (24) and that in a Japanese cohort was 32 years (25). In the USA, the median age was 21years (7), and in Canada, the median age was 24 years (26). Studies have confirmed that patients of different ethnic backgrounds may have dif-

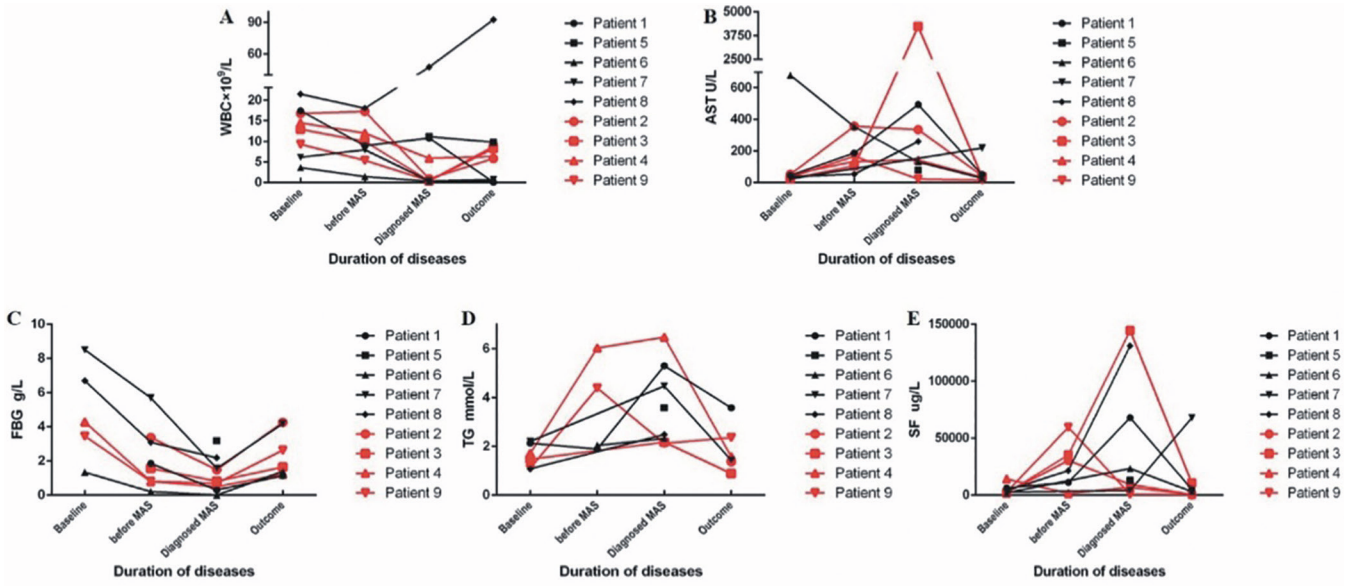


**Fig. 2.** Change of clinical characteristics in AOSD-MAS patients. 2A: WBC; 2B: AST; 2C: FBG; 2D: TG; 2E: SF.

ferent clinical and laboratory results. Japanese and Chinese subjects, are generally older at disease onset than subjects from other races (27). These differences support the hypothesis that a patient' genetic background may influence phenotypic expression (28). The most common symptoms in our study were fever, rash, and arthritis, which were consistent with the typical triad of AOSD (2). In the study by Zeng *et al*. the incidences of fever, rash, and arthralgia were 100%, 89% and 80.33% (29). Although patients can experience joint pain, not all patients develop arthritis. Therefore, the incidence of arthritis is lower than that of arthralgia.

In this study, the incidence of jaundice was higher in AOSD-MAS patients than in AOSD-nonMAS patients and a risk factor for MAS. MAS patients develop jaundice during the disease course. The incidence of jaundice was 6/8 (75%) in MAS patients (30), and AST was higher in AOSD-MAS patients than in AOSD-nonMAS patients. Although ALT and TGs were not significantly different, they were higher in the AOSD-nonMAS group than in the AOSD-MAS group. In a multicentre retrospective study, there was a significant increase in AST (337 (185-684) U/L *vs*. 34 (17-83) U/L, $p<0.001$) (9). Some studies showed differences in splenomegaly, hepatomegaly, lymphadenopathy, sore throat, WBCs, RBCs, PLTs, SF and FBG 12, 20, 21), but in our study did not. AOSD-MAS had fewer unique manifestations than AOSD-nonMAS at the beginning of the disease. High ferritin level is characteristic marker of AOSD and MAS. In our study, 35 AOSD patients (31.5%) were with the SF >10000μg/L and 3 AOSD-MAS (33.3% of AOSD-MAS patients) patients were with the SF >10000μg/L. A Chinese study revealed that ferritin >2000μg/L occupied 36.3% of all AOSD patients (20). SF level in our study consists hyperferritinemia in AOSD and MAS. Those studies compared the data at the time of diagnosis of AOSD, but our data were obtained at the beginning of admission, earlier than the time at diagnosis. Jaundice, and elevated AST, ALT and TGs were related to abnormal liver function. Abnormal liver function is similar to chronic hepatitis, so it leads to the above changes (31, 32). Reportedly, the pathologic images of liver tissue from patients with haemophagocytic syndromes are consistent with those of chronic persistent hepatitis (33). Moreover, this finding suggests that phagocytes destroy liver cells, so jaundice can be used as a predictor of MAS. Some studies have suggested that hepatomegaly (11), AST greater than 120 U/L and TGs greater than 3 mmol/L are independent predictors of MAS (9). An elevated WBC count and MAS were related to death in our retrospective cohort. Five AOSD-MAS patients died, consistent with the high mortality rates of MAS (8, 11, 12, 34). The WBC count is a sensitive inflammatory marker, and a significant increase indicates severe inflammation. Concomitant infection is associated with increased mortality, especially on the background of steroids and DMARDs. Our results showed that the mortality of MAS was 55.6%, and the mortality rate ranges from 5% to 79.6% according to other studies (11, 16, 20). This difference supports the hypothesis that a patients' genetic background may influence phenotypic expression (28), especially compared to western cohort study. The severity of the disease in different patients also causes different mortality rate. And the therapies in different countries are different. The choice of biological agents in China is far less than that in Western countries. In our study, patients died due to MAS, infection and MODS. A previous study suggested that multi-organ involvement at the time of diagnosis and severe infection were predictive of a severe outcome and increased mortality (10, 11). Four MAS patients in this study had a good prognosis, especially Patient 3 (who received biologic agent therapy), and this was considered to be closely related to early detection and early treatment. In MAS, remission is achieved by combination immunosuppression. The early use of high-dose steroids may be successful

alone, but over half of reported adult cases are steroid resistant. The immediate treatment of MAS with 1 g steroid daily for 3-5 days plus 1 g/kg IVIg for 2 days and biologic agents is recommended (16). AOSD is paralleled by a marked increase in the serum levels of pivotal pro-inflammatory cytokines, IL-1, IL-6, IL-18, and TNF-α. And the serum levels of these mediators correlate with disease activity. However, due to the treatment situation in China, we have no way to choose anti-IL-1 agents for AOSD patients. According to the reference, tocilizumab, as an anti-IL-6 agent, has been proven to be effective in the treatment of AOSD (35, 36). We thus chose tocilizumab as the treatment for the patient. It is hoped that effective biological agents can be applied to our patients as soon as possible to improve the survival rate of patients and reduce complications. At that time, we will evaluate the efficacy of related drugs and provide assistance in the selection and optimisation of treatment options. AOSD-MAS patients already had severe liver damage when AOSD was diagnosed. In order to avoid aggravation of liver damage, we reduce the use of hepatotoxic drugs such as methotrexate and azathioprine when choosing therapeutic drugs.

When MAS appears in AOSD, the disease is rampant. Fever changes from spiking during AOSD to persistent high fever, accompanied by abnormal changes in WBCs, PLTs, transaminase, SF and TGs suggesting the occurrence of MAS (20, 37). Patients can fulfil the classification for MAS even if some biomarkers are within the normal reference ranges, which emphasises the need for close monitoring in trends in both the clinical status and laboratory parameters (38). The study showed that blood cells decreased and SF increased, with corresponding changes in the early stages of MAS. Some studies have suggested that changes in blood cells and SF are sensitive indicators of AOSD combined with MAS (6, 11, 17, 20, 21). High TG levels are secondary to decreased lipoprotein lipase activity initiated by increased TNF-α levels (39). TG levels can be reduced in the stage of disease control. A previous report suggested that TGs can be used as the standard for the diagnosis and judgment of MAS (31).

Severe abnormal liver function is very common in patients with MAS, and transaminase is elevated by more than 50% (40). We found that when MAS was diagnosed or patients died, the transaminase and SF levels of some patients showed a downward trend. We should be aware that these findings are indicative not of disease improvement, but rather the opposite levels of bilirubin and transaminase. Due to the massive necrosis of hepatocytes, the processing capacity of bilirubin is progressively reduced, resulting in an increase in bilirubin; moreover, transaminase is maintained at a high level for a long time, resulting in progressive depletion and a decrease in transaminase. This result indicates disease progression. We should analyse other data to identify a patient's condition and try to detect MAS as soon as possible to control the disease.

The limitations of this study include 3 aspects. First, relatively few patients were included because of the short study period and rarity of these disease. Second, to achieve a uniform comparison standard, we selected only the laboratory inspection indicators in our hospital, which may have caused no significant difference between AOSD and MAS in the early stage. Third, some patients did not receive perfect laboratory examinations at the corresponding collection nodes, and there was an insufficient number of patients, so we did not perform a comparison of different severities of MAS. We plan to perform such a comparison when additional data are collected.

AOSD combined with MAS is a disease with high mortality. When patients with AOSD have an unexplained decreased in blood cells, his/or SF continues to increase and severe liver damage occur, clinicians should be aware of the possibility of MAS. When FBG is reduced and phagocytosis is observed in bone biopsy, the possibility of MAS should be considered. More research is needed to enable the early diagnosis and treatment of this disease and to benefit more patients.

**Acknowledgement**

We thank the researchers of the Division of Clinical Research in the First Hospital of Jilin University for their assistance during this study.

**References**

1. STILL GF: On a form of chronic joint disease in children. *Med Chir Trans* 1897; 80: 47-60.9.
2. BYWATERS EG: Still's disease in the adult. *Ann Rheum Dis* 1971; 30: 121-33.
3. BEHRENS EM, BEUKELMAN T, GALLO L *et al*.: Evaluation of the presentation of systemic onset juvenile rheumatoid arthritis: data from the Pennsylvania Systemic Onset Juvenile Arthritis Registry (PASOJAR). *J Rheumatol* 2008; 35: 343-8.
4. FAUTREL B, ZING E, GOLMARD JL *et al*.: Proposal for a new set of classification criteria for adult-onset still disease. *Medicine* (Baltimore) 2002; 81: 194-200.
5. BYWATERS EG, ISDALE IC: The rash of rheumatoid arthritis and Still's disease. *Q J Med* 1956; 25: 377-87.
6. FAUTREL B, LE MOEL G, SAINT-MARCOUX B *et al*.: Diagnostic value of ferritin and glycosylated ferritin in adult onset Still's disease. *J Rheumatol* 2001; 28: 322-9.
7. CUSH JJ, MEDSGER TA, Jr., CHRISTY WC, HERBERT DC, COOPERSTEIN LA: Adult-onset Still's disease. Clinical course and outcome. *Arthritis Rheum* 1987; 30: 186-94.
8. GERFAUD-VALENTIN M, MAUCORT-BOULCH D, HOT A *et al*.: Adult-onset still disease: manifestations, treatment, outcome, and prognostic factors in 57 patients. *Medicine* (Baltimore) 2014; 93: 91-9.
9. WANG R, LI T, YE S *et al*.: Macrophage activation syndrome associated with adult-onset Still's disease: a multicenter retrospective analysis. *Clin Rheumatol* 2020; 39: 2379-86.
10. RUSCITTI P, CIPRIANI P, MASEDU F *et al*.: Adult-onset Still's disease: evaluation of prognostic tools and validation of the systemic score by analysis of 100 cases from three centers. *BMC Med* 2016; 14: 194.
11. BAE CB, JUNG JY, KIM HA, SUH CH: Reactive hemophagocytic syndrome in adult-onset Still disease: clinical features, predictive factors, and prognosis in 21 patients. *Medicine* (Baltimore) 2015; 94: e451.
12. HOT A, TOH ML, COPPERE B *et al*.: Reactive hemophagocytic syndrome in adult-onset Still disease: clinical features and long-term outcome: a case-control study of 8 patients. *Medicine* (Baltimore) 2010; 89: 37-46.
13. FUKAYA S, YASUDA S, HASHIMOTO T *et al*.: Clinical features of haemophagocytic syndrome in patients with systemic autoimmune diseases: analysis of 30 cases. *Rheumatology* (Oxford) 2008; 47: 1686-91.
14. BRISSE E, WOUTERS CH, MATTHYS P: Advances in the pathogenesis of primary and secondary haemophagocytic lymphohistiocytosis: differences and similarities. *Br J Haematol* 2016; 174: 203-17.
15. HENTER JI, SAMUELSSON-HORNE A, ARICO M *et al*.: Treatment of hemophagocytic lymphohistiocytosis with HLH-94 immuno-

chemotherapy and bone marrow transplantation. *Blood* 2002; 100: 2367-73.
16. CARTER SJ, TATTERSALL RS, RAMANAN AV: Macrophage activation syndrome in adults: recent advances in pathophysiology, diagnosis and treatment. *Rheumatology* (Oxford) 2019; 58: 5-17.
17. ZHANG Y, YANG Y, BAI Y, YANG D, XIONG Y, ZENG X: Clinical characteristics and follow-up analysis of adult-onset Still's disease complicated by hemophagocytic lymphohistiocytosis. *Clin Rheumatol* 2016; 35: 1145-51.
18. HENTER JI, HORNE AC, ARICÓ M *et al*.: HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer* 2007; 48: 124-31.
19. FARDET L, GALICIER L, LAMBOTTE O *et al*.: Development and validation of the HScore, a score for the diagnosis of reactive hemophagocytic syndrome. *Arthritis Rheumatol* 2014; 66: 2613-20.
20. YANG XP, WANG M, LI TF, LI W, ZHANG L, LIU SY: Predictive factors and prognosis of macrophage activation syndrome associated with adult-onset Still's disease. *Clin Exp Rheumatol* 2019; 37 (Suppl. 121): S83-8.
21. RUSCITTI P, IACONO D, CICCIA F *et al*.: Macrophage activation syndrome in patients affected by adult-onset still disease: analysis of survival rates and predictive factors in the Gruppo Italiano di Ricerca in Reumatologia Clinica e Sperimentale Cohort. *J Rheumatol* 2018; 45: 864-72.
22. YAMAGUCHI M, OHTA A, TSUNEMATSU T *et al*.: Preliminary criteria for classification of adult Still's disease. *J Rheumatol* 1992; 19: 424-30.
23. GIACOMELLI R, RUSCITTI P, SHOENFELD Y: A comprehensive review on adult onset Still's disease. *J Autoimmun* 2018; 93: 24-36.
24. ZENG T, ZOU YQ, WU MF, YANG CD: Clinical features and prognosis of adult-onset still's disease: 61 cases from China. *J Rheumatol* 2009; 36: 1026-31.
25. OHTA A, YAMAGUCHI M, TSUNEMATSU T *et al*.: Adult Still's disease: a multicenter survey of Japanese patients. *J Rheumatol* 1990; 17: 1058-63.
26. POUCHOT J, SAMPALIS JS, BEAUDET F *et al*.: Adult Still's disease: manifestations, disease course, and outcome in 62 patients. *Medicine* (Baltimore) 1991; 70: 118-36.
27. FRANCHINI S, DAGNA L, SALVO F, AIELLO P, BALDISSERA E, SABBADINI MG: Adult onset Still's disease: clinical presentation in a large cohort of Italian patients. *Clin Exp Rheumatol* 2010; 28: 41-8.
28. JOUNG CI, LEE HS, LEE SW *et al*.: Association between HLA-DR B1 and clinical features of adult onset Still's disease in Korea. *Clin Exp Rheumatol* 2003; 21: 489-92.
29. CHEN PD, YU SL, CHEN S, WENG XH: Retrospective study of 61 patients with adult-onset Still's disease admitted with fever of unknown origin in China. *Clin Rheumatol* 2012; 31: 175-81.
30. LI X, QU B, NIE Y, ZHU G, LI W, MU F: Clinical features of macrophage activation syndrome in the adult northern Chinese population. *Lupus* 2014; 23: 785-92.
31. OKAMOTO M, YAMAGUCHI H, ISOBE Y *et al*.: Analysis of triglyceride value in the diagnosis and treatment response of secondary hemophagocytic syndrome. *Intern Med* 2009; 48: 775-81.
32. BILLIAU AD, ROSKAMS T, VAN DAMME-LOMBAERTS R, MATTHYS P, WOUTERS C: Macrophage activation syndrome: characteristic findings on liver biopsy illustrating the key role of activated, IFN-gamma-producing lymphocytes and IL-6- and TNF-alpha-producing macrophages. *Blood* 2005; 105: 1648-51.
33. FAVARA BE: Hemophagocytic lymphohistiocytosis: a hemophagocytic syndrome. *Semin Diagn Pathol* 1992; 9: 63-74.
34. ARLET JB, LE TH, MARINHO A *et al*.: Reactive haemophagocytic syndrome in adult-onset Still's disease: a report of six patients and a review of the literature. *Ann Rheum Dis* 2006; 65: 1596-601.
35. CAMPOCHIARO C, FARINA N, TOMELLERI A *et al*.: Drug retention rates of biological agents in adult onset Still's disease. *Semin Arthritis Rheum* 2021; 51: 1-6.
36. KANEKO Y, KAMEDA H, IKEDA K *et al*.: Tocilizumab in patients with adult-onset still's disease refractory to glucocorticoid treatment: a randomised, double-blind, placebo-controlled phase III trial. *Ann Rheum Dis* 2018; 77: 1720-9.
37. KIM MJ, AHN EY, HWANG W *et al*.: Association between fever pattern and clinical manifestations of adult-onset Still's disease: unbiased analysis using hierarchical clustering. *Clin Exp Rheumatol* 2018; 36 (Suppl. 115): S74-9.
38. RAVELLI A, MINOIA F, DAVI S *et al*.: 2016 Classification Criteria for Macrophage Activation Syndrome Complicating Systemic Juvenile Idiopathic Arthritis: A European League Against Rheumatism/American College of Rheumatology/Paediatric Rheumatology International Trials Organisation Collaborative Initiative. *Ann Rheum Dis* 2016; 75: 481-9.
39. GEORGE MR: Hemophagocytic lymphohistiocytosis: review of etiologies and management. *J Blood Med* 2014; 5: 69-86.
40. MINOIA F, DAVI S, HORNE A *et al*.: Clinical features, treatment, and outcome of macrophage activation syndrome complicating systemic juvenile idiopathic arthritis: a multinational, multicenter study of 362 patients. *Arthritis Rheumatol* 2014; 66: 3160-9.