Elevated monocyte distribution width in patients with active adult-onset Still's disease: a novel activity indicator

Y.-H. Lin[1], S.-H. Chang[1-3], T.-C. Hsu[1], P.-K. Chen[1,2,4], J.-L. Lan[1,2,5], D.-Y. Chen[1-4,6]

[1]Rheumatology and Immunology Center, China Medical University Hospital, Taichung, Taiwan; [2]College of Medicine, China Medical University, Taichung, Taiwan; [3]PhD Program in Translational Medicine and Rong Hsing Research Center for Translational Medicine, National Chung Hsing University, Taichung, Taiwan; [4]Translational Medicine Laboratory, Rheumatology and Immunology Center, China Medical University Hospital, Taichung, Taiwan; [5]Rheumatic Diseases Research Center, China Medical University Hospital, Taichung, Taiwan; [6]Institute of Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan.

Abstract

Objective

Monocyte distribution width (MDW) correlates with volume modifications of circulating monocytes upon activation. Given the crucial role of monocyte activation in the pathogenesis of adult-onset Still's disease (AOSD), we aimed to examine the associations between MDW and disease activity or inflammatory parameters in this disease.

Methods

In 58 AOSD patients and 95 other patients with coronavirus disease 2019 (COVID-19) as disease control, MDW and complete blood count were determined using a UniCel DxH800 analyser. C-reactive protein (CRP) levels were measured by nephelometry, and ferritin levels by chemiluminescent immunoassay. AOSD activity was assessed using a modified Pouchot score.

Results

MDW was significantly higher in active AOSD patients (median 28.3, interquartile range [IQR] 23.3–32.1) compared with inactive AOSD (19.2, IQR 18.0–20.6, $p<0.001$) or non-severe COVID-19 patients (23.2, IQR 21.0–25.2, $p<0.01$). MDW was positively correlated with AOSD activity scores, CRP, and ferritin levels (all $p<0.001$). Longitudinal follow-up evaluation revealed that median MDW significantly declined (28.3 vs. 18.5, $p<0.001$) along with disease activity, paralleling a decrease in CRP and ferritin levels. Severe COVID-19 and sepsis patients had elevated MDW, which were not different from active AOSD patients. Multivariate analysis revealed MDW as a significant predictor of active AOSD, and MDW threshold at 21.7 could predict an active status with a high sensitivity of 91.3% and specificity of 94.3%.

Conclusion

Elevated MDW and its positive correlation with inflammatory parameters in AOSD patients indicate MDW as a novel activity indicator, with a high MDW value above 21.7 linked to a high probability of active AOSD.

Key words

monocyte distribution width, discriminative marker, activity indicator, COVID-1, adult-onset Still's disease

*Yun-Hsien Lin, MD**
*Shih-Hsin Chang, MD**
Teng-Chieh Hsu, MD
Po-Ku Chen, PhD
Joung-Liang Lan, MD
Der-Yuan Chen, MD, PhD

**These authors contributed equally.*

Please address correspondence to:
Der-Yuan Chen
Rheumatology and Immunology Center,
China Medical University Hospital,
No. 2, Yude Road,
Taichung 40447, Taiwan.
E-mail: dychen1957@gmail.com

Received on February 15, 2023; accepted in revised form on June 12, 2023.

Competing interests: none declared.

Introduction

Adult-onset Still's disease (AOSD) is a rare (1-2), multi-systemic autoinflammatory disorder characterised by fever, skin rash, arthritis, increased acute phase reactants, and hyperferritinaemia (3-5). AOSD has been recognised as an important cause of fever of unknown origin (6). Increased innate immunity and hyperinflammation may produce cytokine storms (7-9) and even cause life-threatening complications like macrophage activation syndrome (MAS) (10) in AOSD. Among the innate immune responses, macrophage activation is the hallmark of AOSD pathogenesis (11). The activated macrophages can stimulate ferritin release, with elevated H-ferritin expressions in lymph nodes and skin correlated with AOSD activity (12-13).

Macrophages can also be activated in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, leading to increased proinflammatory cytokine production, hyperinflammation, and even so-called cytokine storms (14-16). Various monocyte-derived proinflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumour necrosis factor (TNF)-α, were elevated in coronavirus disease 2019 (COVID-19) patients (15-16), suggesting a pathogenic role of monocyte-related immunity in this disease. Although recent studies revealed different cytokine profiles between COVID-19 and active AOSD (17-18), a clear distinction between them is still challenging.

Monocyte distribution width (MDW) is a novel cytometric parameter that correlates with volume modifications of circulating monocytes upon activation (19). Since monocytes are the first responders to infection and display volumetric heterogenicity (20), MDW has been revealed as a valuable sepsis indicator (21-22). Polilli *et al*. found that a combination of MDW at values >22.0 and procalcitonin (PCT) levels >1.0ng/mL was associated with sepsis (23). As in bacterial infections, monocytes may be activated and promote subsequent systemic hyperinflammation in COVID-19 disease (24). MDW could be an inflammatory marker with prognostic significance in this disease (25-27).

Given the pathogenic role of monocyte activation in AOSD (8-9,11), monocyte anisocytosis, quantifiable with MDW, may be a useful marker for disease activity monitoring. Monocyte activation and hyperinflammation are common in both AOSD and COVID-19, which also share similar clinical and laboratory features such as fever, increased acute phase reactants, and hyperferritinaemia (1-3, 17-18, 28-29). It would be interesting to compare the difference in the clinical utility of MDW between these two diseases.

In this pilot study, we analysed MDW, other leukocyte characteristics, and inflammatory parameters; we also investigated the ability of MDW to predict AOSD disease activity and compared that with other markers. Given the similar immune response with hyperinflammation in COVID-19 and AOSD, we enrolled COVID-19 as disease control. Given the diagnosis of AOSD is made by excluding infectious diseases and other rheumatic diseases, we also enrolled sepsis, systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA) as the disease controls. The associations between MDW and inflammatory parameters were also examined in AOSD and COVID-19 patients. Besides, we longitudinally evaluated MDW dynamics during the clinical evolution of AOSD.

Methods

Patients and study design

In this retrospective and single-centre cohort study, fifty-eight AOSD patients fulfilling the Yamaguchi criteria (30) and having a negative result of IgG/IgM for SARS-CoV-2 were consecutively enrolled. Patients with infections, malignancies, or autoimmune diseases were excluded. Systemic disease activity was assessed using a modified Pouchot score (31), with active AOSD defined as systemic activity score ≧4 (32). This score (range, 0–12) assigns one point to each of 12 manifestations: fever, evanescent rash, sore throat, arthralgia or arthritis, myalgia, pleuritis, pericarditis, pneumonitis, lymphadenopathy, hepatomegaly or abnormal liver function, elevated leukocyte count ≧15,000/mm^3, and serum ferritin levels >3000μg/L. Twenty

Table I. Demographic data and laboratory findings in AOSD patients and COVID-19 patients.

Variables	Active AOSD (n=23)	Inactive severe COVID-19 (n=35)	Severe COVID-19 (n=25)	Non-severe COVID-19 (n-70)
Age, years	45.1 ± 17.5$^{\#\#\#,\$\$\$}$	49.5 ± 14.2$^{\#\#\#,\$\$\$}$	72.9 ± 14.7	69.1 ± 23.5
Male, n (%)	5 (21.7%)$^{\#\#\#,\$}$	11 (31.4%)$^{\#\#\#,\$}$	20 (80.0%)	41 (58.6%)
Body mass index	23.4 (20.1-27.0)	23.9 (21.6-28.4)	21.2 (18.7-25.3)	23.2 (20.0-27.0)
MDW	28.3 (23.3-32.1)***,$^{\$\$}$	19.2 (18.0-20.6) $^{\#\#\#,\$\$\$}$	25.1 (22.6-28.8)	23.2 (21.0-25.2)
WBC, x1000/mm^3	11.6 (7.5-16.5)$^{\$\$}$	9.5 (7.7-15.0)$^{\$\$}$	12.7 (7.6-16.9)$^{\$\$}$	6.4 (5.1-9.4)
Neutrophil (%)	87.2 (82.1-91.0)$^{\$\$\$}$	82.3 (72.4-89.2)$^{\$}$	83.6 (67.5-88.7)	72.0 (62.0-82.6)
Lymphocyte (%)	7.4 (3.8-11.7)$^{\$}$	11.1 (5.5-16.5)	8.0 (4.6-17.6)	14.3 (7.3-22.7)
NLR	11.9 (7.0-23.2)$^{\$\$}$	7.4 (4.4-16.1)	10.6 (4.0-19.0)	5.3 (2.7-11.6)
CRP levels, mg/dL	5.7 (2.4-11.8)***	1.2 (0.6-2.4)$^{\#\#\#,\$}$	8.1 (3.9-15.1)$^{\$\$}$	3.1 (1.2-6.7)
Ferritin levels, ng/mL	2851 (899-4598)***,$^{\$\$\$}$	145 (119-224)$^{\#\#\#}$	705 (218-987)	236 (124-389)
Drugs at study entry	23 (100%)	21 (60.0%)	24 (96.0%)	20 (28.6%)
Corticosteroids, n (%)				
Methotrexate, n (%)	19 (82.6%)	20 (57.1%)	0 (0.0%)	0 (0.0%)
HCQ, n (%)	16 (69.6%)	18 (51.4%)	0 (0.0%)	0 (0.0%)
Cyclosporine, n (%)	8 (34.8%)	3 (8.6%)	0 (0.0%)	0 (0.0%)
IL-6R inhibitor, n (%)	8 (34.8%)	2 (5.7%)	10 (40.0%)	0 (0.0%)
Mortality rate, n (%)	1 (4.3%)	0 (0.0%)	14 (56.0%)	0 (0.0%)

Data are presented as mean ± SD, number (%), or median (25th -75th quartile range); AOSD: adult-onset Still's disease; COVID-19: coronavirus disease 2019; WBC: white blood cell count; MDW: monocyte distribution width; NLR: neutrophil-to-lymphocyte ratio; CRP: C-reactive protein; HCQ: hydroxychloroquine; IL-6R: interleukin-6 receptor.
*p<0.05, **p<0.001, *vs*. inactive AOSD, as determined by Mann-Whitney U-test.
$^{\#}p$<0.05, $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001, *vs*. severe COVID-19, as determined by Mann-Whitney U-test.
$^{\$}p$<0.05, $^{\$\$}p$<0.001, *vs*. non-severe COVID-19, as determined by Mann-Whitney U-test.

patients fulfilling the 1997 revised criteria for SLE (33), twenty-two patients fulfilling the 2010 classification criteria for RA (34), and twenty-three patients fulfilling the 2015 clinical criteria for sepsis (35) were included as AOSD disease controls. SLE disease activity was determined using calculating the SLE disease activity index-2K score (SLEDAI-2K) (36), and active SLE was defined as a SLEDAI-2K score of at least 6. RA disease activity was assessed using the 28-joint disease activity score-erythrocyte sedimentation rate (DAS28-ESR) (37), and active status was defined as DAS28 ≧3.2.

We also enrolled 95 Taiwanese patients with laboratory-confirmed COVID-19, which were positive results of polymerase-chain-reaction assay of nasal or pharyngeal swab specimens. According to the WHO-China Joint Mission on COVID-19, the severity of COVID-19 patients was divided into mild (constitutional symptoms without pneumonia), moderate (COVID-19 pneumonia), and severe (severe dyspnoea requiring mechanical ventilation, shock, other organs failure requiring intensive care, or mortality) (38); mild or moderate COVID-19 was considered non-severe. The hospital's Institutional Review Board approved this study (CMUH110-REC2-106), and the written consent was waived because this is a retrospective analysis.

The patients' data reviewed herein included demographics, medical history, clinical and laboratory assessment results, and the use of medications, including conventional synthetic disease-modifying anti-rheumatic drugs (cs-DMARDs) and the biologic DMARDs.

Determination of MDW and other inflammatory parameters

In this study, with a non-interventional design, MDW was assessed along with routine complete blood cell (CBC) count testing. The whole blood samples were withdrawn and collected in phlebotomy tubes, which contained di-potassium ethylenediaminetetraacetic acid (K2 EDTA) anticoagulant. They were then analysed using the UniCel DxH 800 haematology analyser (Beckman Coulter, Inc. Brea, CA, USA). MDW was determined in conjunction with routine complete blood count testing within 2 hours of blood collection. MDW was automatically calculated as the standard deviation (SD) of monocyte volume divided by the mean monocyte volume and multiplied by 100 to present value as a percentage, as previously reported (21-23). The neutrophil-to-lymphocyte ratio (NLR) was calculated as preoperative neutrophil count/lymphocyte count in peripheral blood (39).

Determination of serum levels of C-reactive protein (CRP) and ferritin

All serum samples were obtained through centrifugation and then assayed immediately or stored at -20°C until investigation. According to the manufacturer's instructions, CRP levels were measured by nephelometry (Behring, Behring, Germany) and serum ferritin light-chain levels by ELISA (eBioscience, San Diego, CA, USA).

Determination of serum levels of IL-1β and IL-18 in AOSD patients

The ELISA kits for IL-1β (RayBiotech Inc., Norcross, GA, USA) and IL-18 (Medical & Biology Laboratories Co, Ltd., Naka-Ku, Nagoya, Japan) were used to determine the serum levels of inflammasome-related cytokines, according to the manufacturer's instructions.

Statistical analysis

The results were presented as the mean ± SD or median (interquartile range). We performed a chi-squared test to examine the between-group difference of categorical variables. The Kruskal-Wallis test was used to compare the levels

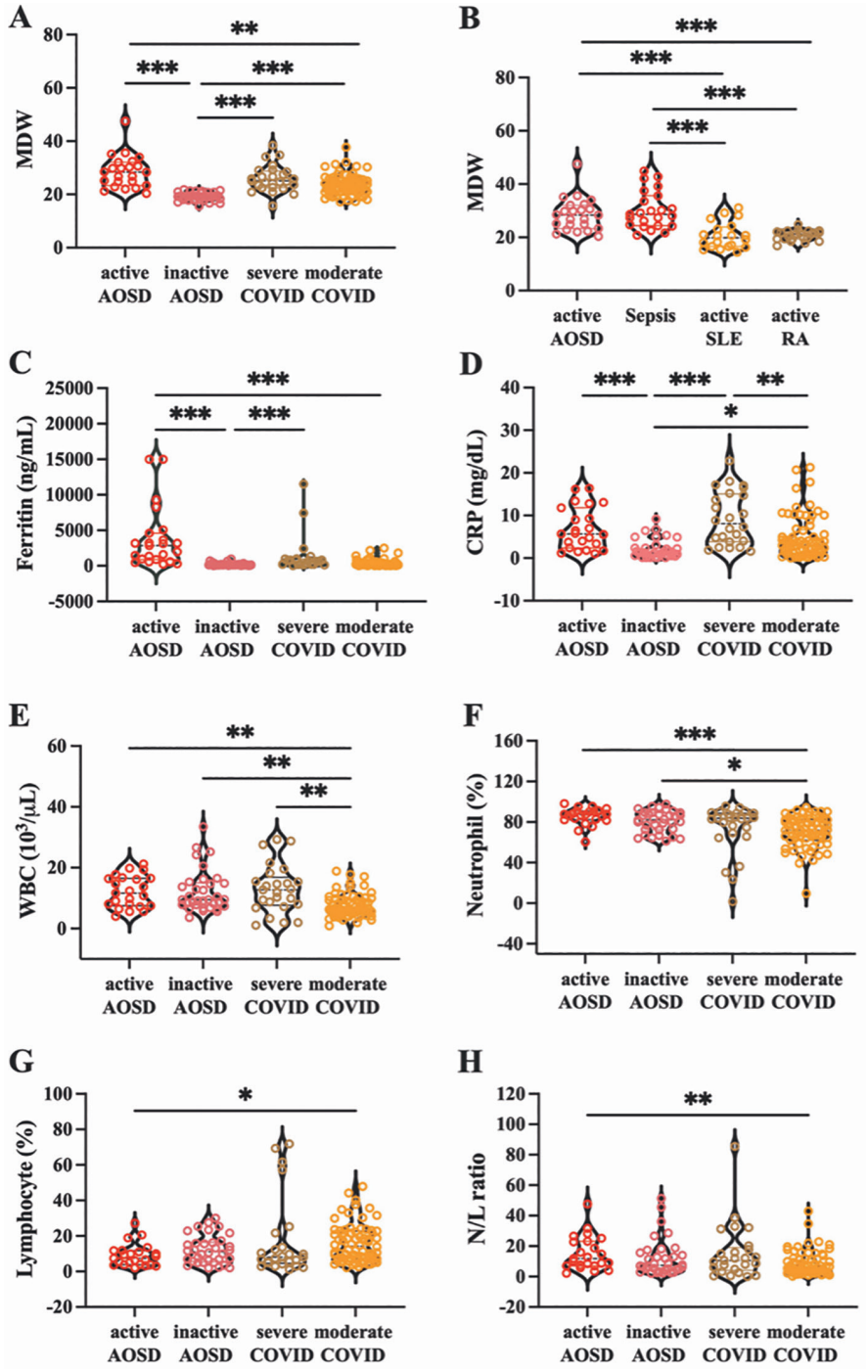


Fig. 1. Comparison of MDW, leukocyte parameters, CRP, and ferritin levels among AOSD and COVID-19 patients.
The difference in values of (**A, B**) MDW, (**C**) ferritin levels, (**D**) CRP, (**E**) WBC count, (**F**) neutrophil percentage, (**G**) lymphocyte percentage, and (**H**) NLR among the enrolled participants.
AOSD: adult-onset Still's disease; COVID-19: coronavirus disease 2019; SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; MDW: monocyte distribution width; CRP: C-reactive protein; WBC: white blood cells; NLR: neutrophil-to-lymphocyte ratio.
Data are presented as box-plot diagrams, with the box encompassing the 25th percentile (lower bar) to the 75th percentile (upper bar). The horizontal line within the box indicates median value respectively for each group. *p<0.05, **p<0.01, ***p<0.001, determined by Mann-Whitney U-test.

of MDW, CRP, ferritin, and leukocyte parameters. When this test showed a significant difference, the exact p-value was determined using the Mann-Whitney U-test. The correlation coefficient was obtained through Spearman's rank correlation test. A multivariate logistic regression model was used to evaluate the powers of MDW, leukocyte parameters, and inflammatory markers to predict an active state of AOSD or mortality in COVID-19. The receiver-operating characteristic (ROC) curve analysis was performed to determine the area under the ROC curve (AUC), sensitivity, and specificity using MedCalc v.14. The missing values were excluded from the statistical analysis. A two-sided probability of less than 0.05 was considered significant.

Results

Clinical characteristics of AOSD patients and COVID-19 patients

Among the enrolled patients, 23 (39.7%) had active AOSD, and 25 (26.3%) had severe COVID-19. Patients with COVID-19, particularly severe COVID-19 (72.9±14.7 years), were older than those with active (45.1±17.5 years) or inactive AOSD (49.5±14.2 years); besides, they were predominantly male (64.2% *vs*. 27.6%, p<0.05, Table I). As illustrated in Supplementary Table S1, sepsis patients were significantly older than AOSD, RA, or SLE patients. A significantly higher proportion of males was also observed in sepsis patients than in active AOSD, SLE, and RA patients.
Fever was the most common manifestation in patients with sepsis, active AOSD, or COVID-19 (100%, 91.3%, and 77.9%, respectively). The distinct characteristics of AOSD patients included a higher proportion of skin rash (87.0% *vs*. 8.4%, p<0.001), arthralgia or arthritis (69.6% *vs*. 17.9%, p<0.01), sore throat (60.9% *vs*. 32.6%, p<0.05), and liver dysfunction (47.8% *vs*. 11.6%, p<0.05), but a lower proportion of pulmonary involvement (4.3% *vs*. 57.9%, p<0.01).

Comparison of MDW, leukocyte parameters, CRP and ferritin levels among all the enrolled patients

As shown in Figure 1A, MDW values were significantly higher in active AOSD patients compared with inactive AOSD patients and non-severe COVID-19 (p<0.001 and p<0.01, respectively). Like active AOSD patients, severe or non-severe COVID-19 patients also had significantly higher MDW than inactive AOSD patients; however,

there was no significant difference in MDW between severe and non-severe COVID-19 patients. Compared with the disease control of AOSD, MDW values were also significantly higher in active AOSD patients than in active SLE or active RA patients (both $p<0.001$, Fig. 1B). However, there was no significant difference in MDW values between active AOSD and sepsis patients. Sepsis patients also had significantly higher MDW values than active SLE or RA patients (both $p<0.001$) (Fig. 1B and Suppl. Table S1).

As illustrated in Figure 1C-1H, active AOSD patients had significantly higher ferritin levels and CRP than inactive AOSD patients. However, there was no significant difference in white blood cell (WBC) count, percentages of neutrophils or lymphocytes, or NLR between active and inactive AOSD patients. Severe COVID-19 patients had significantly higher CRP and ferritin levels than inactive AOSD patients and significantly higher CRP levels and WBC count than non-severe COVID-19 patients. However, there was no significant difference in MDW, WBC count, percentage of neutrophil or lymphocytes, NLR, and serum levels of CRP or ferritin between active AOSD and severe COVID-19 patients.

Correlation between MDW and disease activity or inflammatory markers

As shown in Figure 2A, MDW values were positively correlated with activity scores ($p<0.001$), ferritin levels ($p<0.001$), CRP levels ($p<0.001$), neutrophil percentage ($p<0.01$), and NLR ($p<0.01$), while were negatively correlated with lymphocyte percentage ($p<0.01$) in AOSD patients. Among COVID-19 patients, MDW values were positively correlated with CRP levels (i<0.01) (Fig. 2B).

Based on the link between increased MDW and inflammasome activation in sepsis patients (40), we also examined the correlation between MDW values and inflammasome-related cytokines, including IL-1β and IL-18, in AOSD patients. Our results revealed a positive correlation between MDW and IL-1β (r=0.728, $p<0.001$) as well as IL-18 (r= 0.701, $p<0.001$) in 24 patients with AOSD.

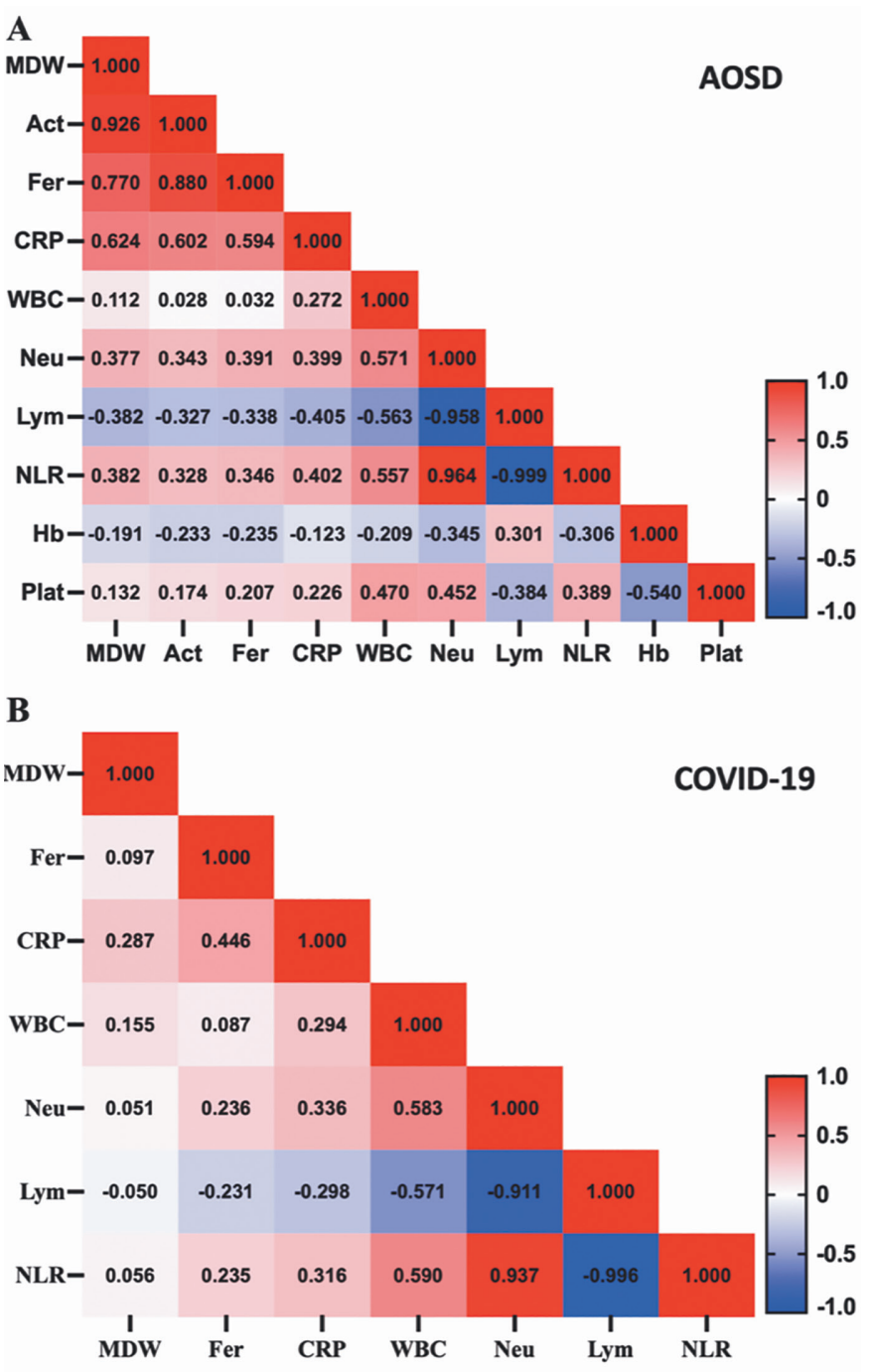


Fig. 2. Correlation matrix between MDW and inflammatory parameters in patients with (**A**) AOSD or (**B**) COVID-19. The correlation coefficient was obtained through the Spearman's rank correlation test. MDW: monocyte distribution width; CRP: C-reactive protein; AOSD: adult-onset Still's disease; COVID-19: coronavirus disease 2019.

Changes in MDW, disease activity, and inflammatory markers in AOSD patients

As shown in Figure 3, MDW significantly declined (median 28.3, IQR 23.3-32.1 *vs*. 18.5, IQR 17.6–19.9, $p<0.001$) in AOSD patients after 6–12 months of therapy, paralleling the decreases in systemic activity scores (median 5.0, IQR 5.0–6.0 *vs*. 2.0, IQR 1.0–2.0, $p<0.001$), CRP levels (median 5.8 mg/dL, IQR 2.9–12.3 mg/dL *vs*. 0.20 mg/dL, IQR 0.04–0.64 mg/dL, $p<0.001$), and ferritin levels (median 2851 ng/mL, IQR 899–4598 ng/mL *vs*. 154 ng/mL,

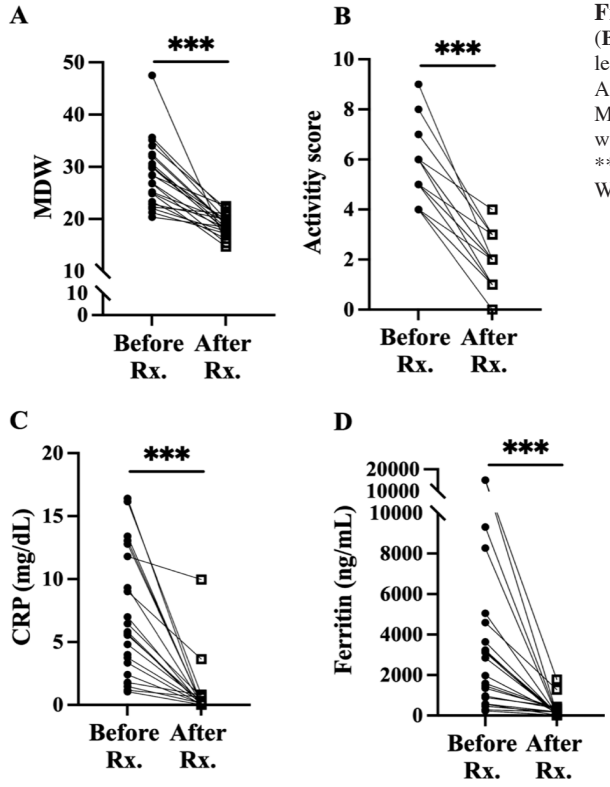


Fig. 3. The change in (**A**) MDW, (**B**) activity scores, (**C**) CRP levels, and (**D**) ferritin levels in AOSD patients after therapy. MDW: monocyte distribution width; CRP: C-reactive protein. ***$p<0.001$, determined by Wilcoxon signed rank test.

Table II. Logistic regression analysis of MDW, leukocyte parameters, and inflammatory parameters to predict active state in patients with adult-onset Still's disease.

Baseline variables	Univariate model			Multivariate model		
	OR	95%CI	*p*-value	OR	95%CI	*p*-value
Age, years	0.98	(0.95-1.02)	0.297			
Gender						
Male		ref.				
Female	1.65	(0.49-5.60)	0.422			
MDW	7.27	(1.42-37.2)	0.017	7.68	(1.46-40.37)	0.016
WBC count, x1000/mm3	1.00	(0.92-1.09)	0.979			
Neutrophil (%)	1.06	(1.00-1.13)	0.068			
Lymphocyte (%)	0.94	(0.86-1.01)	0.107			
NLR	1.02	(0.98-1.07)	0.350			
Haemoglobin, g/dL	0.88	(0.64-1.21)	0.420			
Platelet, x1000/mm3	1.00	(1.00-1.01)	0.421			
Ferritin levels, ng/mL	1.01	(1.00-1.01)	0.002			
CRP levels, mg/dL	1.48	(1.18-1.86)	0.001			

OR: odds ratio; 95% CI: 95% confidence interval; MDW: monocyte distribution width; WBC: white blood cell count; NLR: neutrophil-to-lymphocyte ratio; CRP: C-reactive protein.
Variables in multivariable logistic regression: age, gender, baseline disease activity score and inflammatory parameters.

IQR 76–333 ng/mL, $p<0.001$). In ten patients with untreated active AOSD, MDW also significantly declined (median 28.4, IQR 26.4–32.2 *vs*. 19.1, IQR 17.2–20.3, $p<0.01$) after 6–12 months of combination therapy with corticosteroids and DMARDs, paralleling the decreases in systemic activity scores (median 6.0, IQR 5.0–6.3 *vs*. 2.0, IQR 1.8–3.0, $p<0.01$).

Logistic regression analysis for predicting active state in AOSD patients

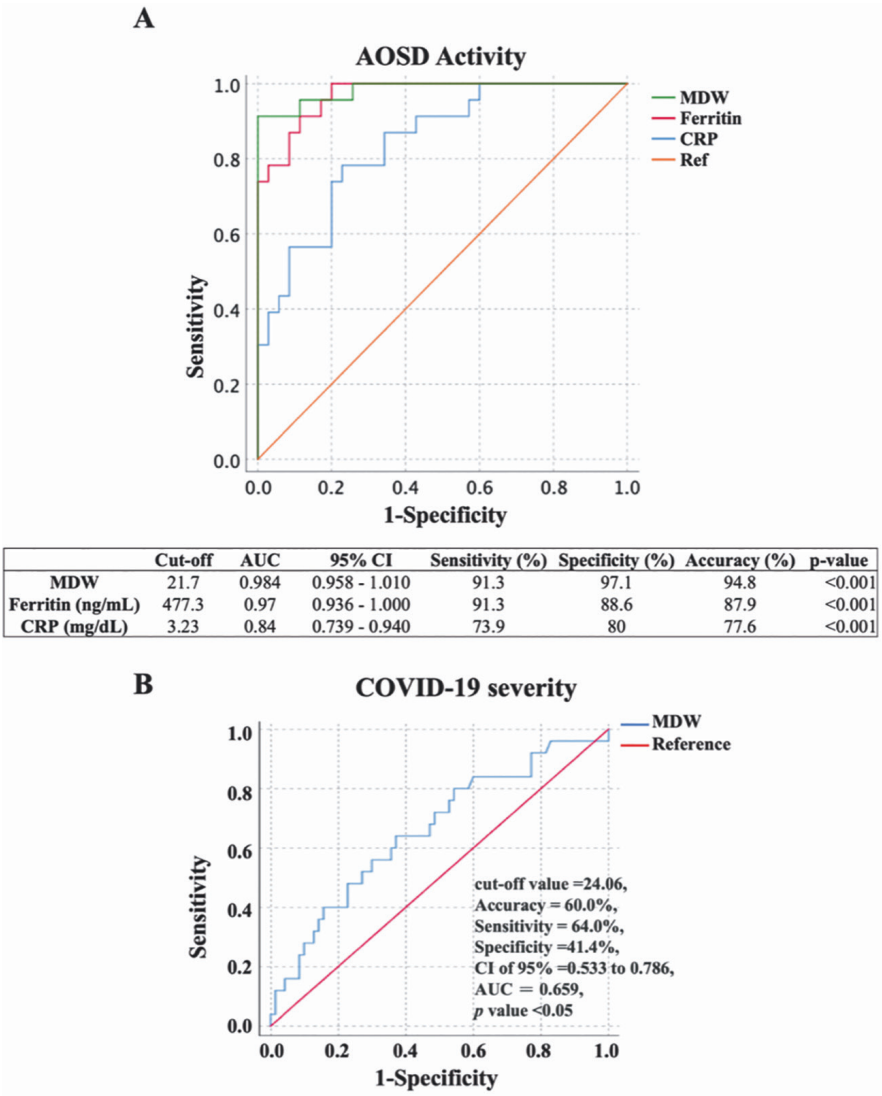
As illustrated in Table II, the univariate regression analysis identified that baseline MDW, ferritin, and CRP levels were significant predictors of active AOSD. The multivariate regression analysis also identified baseline MDW as a significant predictor of an active state of AOSD. Regarding the prediction for the severity of COVID-19 patients, the univariate regression analysis identified that baseline MDW, CRP levels, WBC counts, and NLR were significant predictors; however multivariate regression analysis did not reveal any significant predictor for COVID-19 severity.

Receiver operating characteristic (ROC) curve analysis

Using ROC curve analysis, we evaluated the performance of MDW, ferritin levels, and CRP in discriminating between active and inactive AOSD patients. As shown in Figure 4A, the prediction of AOSD activity by MDW showed a better performance (AUC 0.984) than ferritin (AUC 0.97) and CRP (AUC 0.84). MDW at the cut-off value of 21.7 revealed the highest predictive power, with sensitivity of 91.3%, specificity of 97.1%, and accuracy of 94.8% ($p<0.001$). The optimal cut-off value of MDW for predicting COVID-19 severity was 24.1, with an AUC of 0.659, 64.0% sensitivity, 41.4% specificity, and 60.0% accuracy ($p<0.05$) (Fig. 4B).

Discussion

With the advent of cytometry-based analysis of cell volume distribution within a single group of immune cells (24), MDW can be determined along with routine CBC evaluation. The present study is the first to demonstrate elevated MDW in both active AOSD patients and severe COVID-19 patients relative to inactive AOSD patients. There was also a positive correlation between MDW values and disease activity, including systemic activity scores, serum ferritin, and CRP levels, in AOSD patients. The multivariate logistic regression analysis revealed MDW as a significant predictor of active AOSD. The ROC curve analysis also demonstrated that the MDW threshold at 21.7 could predict an active status of AOSD with a high sensitivity of 91.3% and specificity of 94.3%. Besides, the decrease in MDW paralleled the improvement in disease activity in AOSD patients. These observations suggest that MDW is a useful predictor



	Cut-off	AUC	95% CI	Sensitivity (%)	Specificity (%)	Accuracy (%)	p-value
MDW	21.7	0.984	0.958 - 1.010	91.3	97.1	94.8	<0.001
Ferritin (ng/mL)	477.3	0.97	0.936 - 1.000	91.3	88.6	87.9	<0.001
CRP (mg/dL)	3.23	0.84	0.739 - 0.940	73.9	80	77.6	<0.001

Fig. 4. ROC curves analysis of MDW and inflammatory markers for predicting (**A**) AOSD activity or (**B**) COVID-19 severity.
ROC: receiver-operating characteristic; MDW: monocyte distribution width; CRP: C-reactive protein; AUC: area under ROC curve; 95% CI: 95% confidence interval; AOSD: adult-onset Still's disease; COVID-19: coronavirus disease 2019.

of an active state of immune response in AOSD.

It is well known that monocyte activation plays a crucial role in innate immunity and hyperinflammation in AOSD (9-11). Melnikov *et al*. reported that activated monocytes could transport circulating CRP through monocyte-derived exosomes to maintain inflammatory response (41). We thus speculated a link between MDW, a monocyte activation marker, and AOSD's disease activity. Our results showed significantly higher MDW in active AOSD patients than in inactive AOSD patients. The MDW values were positively correlated with AOSD disease activity and inflammatory parameters, including serum ferritin and CRP levels. Besides, there was a significant decrease in MDW after effective therapy in AOSD patients, paralleling the clinical remission and the decrease in inflammatory parameters. Iwamoto et al. similarly revealed that elevated ferritin from macrophage activation was correlated with the disease activity of AOSD (42). Herein, the multivariate logistic regression analysis revealed MDW as the most discriminatory parameter among leukocyte markers and inflammatory parameters for the active status of AOSD. Using ROC curve analysis, MDW was also the most discriminatory parameter among all the studied activity indicators: a high MDW value above 21.7, set as an AUC-ROC threshold, was linked to a high probability of active AOSD, with a high sensitivity, specificity, and accuracy. Hence, MDW could be a useful, novel activity indicator for AOSD.

Given the diagnosis of AOSD is made by excluding infectious diseases and other rheumatic diseases (30), we enrolled sepsis, SLE, and RA as disease controls of AOSD. Consistent with the findings of previous studies (22-23), we reveal an elevated MDW in sepsis patients. Our results also showed no significant difference in MDW values between sepsis patients and active AOSD patients. Compared with patients with active SLE or RA, we show significantly higher values of MDW in active AOSD patients, suggesting the role of MDW for discriminating AOSD from other rheumatic diseases.

As in active AOSD, activated monocytes promote proinflammatory cytokine production and may even cause the so-called cytokine storm in COVID-19 (14-15). Elevated MDW has been reported in COVID-19 patients, particularly in severe patients (25-27), and MDW was revealed as a novel inflammatory marker in COVID-19 patients (26-27). Accordingly, we observed a positive correlation between MDW and CRP levels in COVID-19 patients. Besides, a high MDW value above 24.1, an AUC-ROC threshold, was linked to a high probability of severe COVID-19. The result resonates with recent reports showing that MDW correlated with the clinical severity of COVID-19 (26). Arya *et al*. similarly reported that a cut-off value (24.0) of MDW could help identify multisystemic inflammatory syndrome in children, the severe form of COVID-19 (43). Since monocyte activation with hyperinflammation is common in both AOSD and COVID-19, we revealed no significant difference in MDW between active AOSD patients and severe COVID-19 patients.

The mechanisms underlying the increased MDW in AOSD remain unclear. Viewing that inflammasome activation plays a pivotal role in the transition from localised infection to sepsis, Eisinger *et al*. investigated the effects of

inflammasome activation on the monocyte size distribution and MDW by using *in vitro* sepsis model (40). They revealed that inflammasome stimulation by lipopolysaccharide would activate caspase-1, which cleaved gasdermin-D to form oligomeric pores in the plasma membrane of monocytes and facilitated the release of IL-1β and IL-18. The increased MDW in sepsis patients may reflect the presence of swollen monocytes undergoing inflammasome-related pyroptosis (40). Our study similarly revealed a positive correlation between MDW and serum levels of inflammasome-related IL-1β and IL-18 in patients with AOSD, an inflammasome-related autoinflammatory disease (44) with the elevated levels of gasdermin-D (45). Nevertheless, the exact mechanism for the increased MDW in active AOSD awaits further investigation.

Despite the novel findings, there are some limitations in our study. The retrospective nature of our study did not allow for obtaining all the needed information from the enrolled patients. The lack of statistical significance in the multivariate regression analysis of MDW for predicting severity or mortality could be due to the small sample size of severe COVID-19 patients. Besides, the selection of the cut-off level of MDW for predicting an active state of AOSD awaits further external validation. Therefore, there is a need for future prospective studies which enrol more AOSD patients and include those with sepsis (46) as another disease control.

Conclusion

MDW was elevated and positively correlated with disease activity and inflammatory parameters in AOSD, showing that MDW might be a novel activity indicator. Because MDW is a useful and easily accessible parameter, MDW testing could help timely adjust the therapeutic agents for AOSD patients in clinical practice.

References

1. MAGADUR-JOLY G, BILLAUD E, BARRIER JH *et al*.: Epidemiology of adult Still's disease: estimate of the incidence by a retrospective study in west France. *Ann Rheum Dis* 1995; 54(7): 587-90. https://doi.org/10.1136/ard.54.7.587
2. WAKAI K, OHTA A, TAMAKOSHI A *et al*.: Estimated prevalence and incidence of adult Still's disease: findings by a nationwide epidemiological survey in Japan. *J Epidemiol* 1997; 7(4): 221-5.
3. NARULA N, NARULA T, ABRIL A: Seizing the clinical presentation in adult-onset Still's disease. An extensive literature reviews. *Autoimmun Rev* 2015; 14: 472-477. https://doi.org/10.1016/j.autrev.2015.01.007
4. KADAVATH S, EFTHIMIOU P: Adult-onset Still's disease-pathogenesis, clinical manifestations, and new treatment options. *Ann Med* 2015; 47(1): 6-14. https://doi.org/10.3109/07853890.2014.971052
5. HU QY, ZENG T, SUN CY *et al*.: Clinical features and current treatments of adult-onset Still's disease: a multicentre survey of 517 patients in China. *Clin Exp Rheumatol* 2019; 37 (Suppl. 121): S52-7.
6. LARKIN JG, STURROCK RD: Adult Still's disease. A new consideration in pyrexia of unknown origin. *Scott Med J* 1983; 28(3): 255-8. https://doi.org/10.1177/003693308302800308
7. GERFAUD-VALENTIN M, JAMILLOUX Y, IWAZ J, SEVE P: Adult-onset Still's disease. *Autoimmun Rev* 2014; 13(7): 708-22. https://doi.org/10.1016/j.autrev.2014.01.058
8. FEIST E, MITROVIC S, FAUTREL B: Mechanisms, biomarkers and targets for adult-onset Still's disease. *Nat Rev Rheumatol*.2018; 14(10): 603-18. https://doi.org/10.1038/s41584-018-0081-x
9. WANG MY, JIA JC, YANG CD, HU QY: Pathogenesis, disease course, and prognosis of adult-onset Still's disease: an update and review. *Chin Med J* (Engl) 2019; 132(23): 2856-64. https://doi.org/10.1097/cm9.0000000000000538
10. LENERT A, YAO Q: Macrophage activation syndrome complicating adult-onset Still's disease: a single center case series and comparison with literature. *Semin Arthritis Rheum* 2016; 45: 711-6. https://doi.org/10.1016/j.semarthrit.2015.11.002
11. CHEN PK, CHEN DY: An update on the pathogenic role of macrophages in adult-onset Still's disease and its implication in clinical manifestations and novel therapeutics. *J Immunol Res* 2021; 2021: 8998358. https://doi.org/10.1155/2021/8998358
12. RUSCITTI P, CICCIA F, CIPRIANI P *et al*.: The CD68(+)/H-ferritin(+) cells colonize the lymph nodes of the patients with adult onset Still's disease and are associated with increased extracellular level of H-ferritin in the same tissue: correlation with disease severity and implication for pathogenesis. *Clin Exp Immunol* 2016; 183(3): 397-404. https://doi.org/10.1111/cei.12738
13. RUSCITTI P, CIPRIANI P, CICCIA F *et al*.: H-ferritin and CD68(+) /H-ferritin(+) monocytes/macrophages are increased in the skin of adult-onset Still's disease patients and correlate with the multi-visceral involvement of the disease. *Clin Exp Immunol* 2016; 186(1): 30-8. https://doi.org/10.1111/cei.12826
14. VANINOV N: In the eye of the COVID-19 cytokine storm. *Nat Rev Immunol* 2020; 20(5): 277. https://doi.org/10.1038/s41577-020-0305-6
15. BHASKAR S, SINHA A, BANACH M *et al*.: Cytokine storm in COVID-19-immunopathological mechanisms, clinical considerations, and therapeutic approaches: The REPROGRAM Consortium Position Paper. *Front Immunol* 2020; 11: 1648. https://doi.org/10.3389/fimmu.2020.01648
16. TANG Y, LIU J, ZHANG D, XU Z, JI J, WEN C: Cytokine Storm in COVID-19: The current evidence and treatment strategies. *front immunol* 2020; 11: 1708. https://doi.org/10.3389/fimmu.2020.01708
17. MENG J, MA Y, JIA J *et al*.: Cytokine storm in Coronavirus disease 2019 and adult-onset Still's disease: similarities and differences. *Front Immunol* 2021; 11: 603389. https://doi.org/10.3389/fimmu.2020.603389
18. CHEN PK, LAN JL, HUANG PH *et al*.: Interleukin-18 is a potential biomarker to discriminate active adult-onset Still's disease from COVID-19. *Front Immunol* 2021; 12: 719544. https://doi.org/10.3389/fimmu.2021.719544
19. SPRANGERS S, DE VRIES TJ, EVERTS V: Monocyte heterogeneity: consequences for monocyte-derived immune cells. *J Immunol Res* 2016; 2016: 1475435. https://doi.org/10.1155/2016/1475435
20. WONG KL, YEAP WH, TAI JJ, ONG SM, DANG TM, WONG SC: The three human monocyte subsets: implications for health and disease. *Immunol Res* 2012; 53(1-3): 41-57. https://doi.org/10.1007/s12026-012-8297-3
21. CROUSER ED, PARRILLO JE, SEYMOUR C *et al*.: Improved early detection of sepsis in the ED with a novel monocyte distribution width biomarker. *Chest* 2017; 152(3): 518-526. https://doi.org/10.1016/j.chest.2017.05.039
22. CROUSER ED, PARRILLO JE, SEYMOUR CW *et al*.: Monocyte Distribution Width: A Novel Indicator of Sepsis-2 and Sepsis-3 in High-Risk Emergency Department Patients. *Crit Care Med* 2019; 47(8): 1018-1025. https://doi.org/10.1097/CCM.0000000000003799
23. POLILLI E, SOZIO F, FRATTARI A *et al*.: Comparison of monocyte distribution width (MDW) and procalcitonin for early recognition of sepsis. *PLoS One* 2020; 15(1): e0227300. https://doi.org/10.1371/journal.pone.0227300
24. MARTINEZ FO, COMBES TW, ORSENIGO F, GORDON S: Monocyte activation in systemic Covid-19 infection: Assay and rationale. *EBioMedicine* 2020; 59: 102964. https://doi.org/10.1016/j.ebiom.2020.102964
25. LIPPI G, SANCHIS-GOMAR F, HENRY BM: Pooled analysis of monocyte distribution width in subjects with SARS-CoV-2 infection. *Int J Lab Hematol* 2021; 43(4): O161-O163. https://doi.org/10.1111/ijlh.13482
26. RIVA G, CASTELLANO S, NASILLO V *et al*.: Monocyte distribution width (MDW) as novel inflammatory marker with prognostic significance in COVID-19 patients. *Sci Rep* 2021; 11(1): 12716. https://doi.org/10.1038/s41598-021-92236-6
27. ALSUWAIDI L, Al HEIALY S, SHAIKH N *et al*.: Monocyte distribution width as a novel sepsis indicator in COVID-19 patients. *BMC Infect Dis* 2022; 22(1): 27. https://doi.org/10.1186/s12879-021-07016-4
28. HUANG C, WANG Y, LI X *et al*.: Clinical fea-

tures of patients infected with 2019 novel coronavirus in Wuhan, China [published correction appears in *Lancet* 2020 Jan 30]. *Lancet* 2020; 395(10223): 497-506. https://doi.org/10.1016/S0140-6736(20)30183-5
29. GUAN W, NI Z, HU Y, *et al*.: Clinical characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 2020; 382: 1708-20. https://doi.org/10.1056/nejmoa2002032
30. YAMAGUCHI M, OHTA A, TSUNEMATSU T *et al*.: Preliminary criteria for classification of adult Still's disease. *J Rheumatol* 1992; 19(3): 424-30.
31. RAU M, SCHILLER M, KRIENKE S, HEYDER P, LORENZ H, BLANK N: Clinical manifestations but not cytokine profiles differentiate adult-onset Still's disease and sepsis. *J Rheumatol* 2010; 37(11): 2369-76. https://doi.org/10.3899/jrheum.100247
32. AHN MH, HAN JH, CHWAE YJ *et al*.: Neutrophil extracellular traps may contribute to the pathogenesis in adult-onset Still disease. *J Rheumatol*.2019; 46(12): 1560-9. https://doi.org/10.3899/jrheum.181058
33. HOCHBERG MC: Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.
34. ALETAHA D, NEOGI T, SILMAN AJ *et al*.: 2010 rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010; 69: 1580-8.
35. SEYMOUR CW, LIU V, IWASHYNA TJ *et al*.: Assessment of clinical criteria for sepsis. *JAMA* 2016; 315(8): 762-74. https://doi.org/10.1001/jama.2016.0288
36. BOMBARIDIER C, GLADMAN DD, UROWITZ MB, CARON D, CHANG CH: The Committee on Prognosis Studies in SLE. Derivation of the SLEDAI: a disease activity index for lupus patients. *Arthritis Rheum* 1992; 35: 630-40.
37. PREVOO ML, VAN 'T HOF MA, KUPER HH, VAN LEEUWEN MA, VAN DE PUTTE LB, VAN RIEL PL: Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995; 38: 44-8. https://doi.org/10.1002/art.1780380107
38. World Health Organization (2020). Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19). https://www.who.int/docs/defaultsource/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf.
39. ZAHOREC R: Neutrophil-to-lymphocyte ratio, past, present and future perspectives. *Bratisl Lek Listy* 2021; 122(7): 474-88. https://doi.org/10.4149/BLL_2021_078
40. EISINGER GJ, OSMAN W, PRATHER ER *et al*.: Inflammsome activation in an intro sepsis model recapitulates increased monocyte distribution width seen in patients with sepsis. *Crit care Explor* 2022; 4(2): e0631. https://doi.org/10.1097/cce.0000000000000631
41. MELNIKOV I, KOZLOV S, SABUROVA O *et al*.: CRP is transported by monocytes and monocyte-derived exosomes in the blood of patients with coronary artery disease. *Biomedicines* 2020; 8(10): 435. https://doi.org/10.3390/biomedicines8100435
42. KIRINO Y, KAWAGUCHI Y, TADA Y *et al*.: Beneficial use of serum ferritin and heme oxygenase-1 as biomarkers in adult-onset Still's disease: A multicenter retrospective study. *Mod Rheumatol* 2018; 28(5): 858-64. https://doi.org/10.1080/14397595.2017.1422231
43. YONKER LM, BADAKI-MAKUN O, ARYA P *et al*.: Monocyte anisocytosis increases during multisystem inflammatory syndrome in children with cardiovascular complications [published correction appears in *BMC Infect Dis* 2022; 22(1): 595]. *BMC Infect Dis* 2022; 22(1): 563. https://doi.org/10.1186/s12879-022-07526-9
44. HSIEH CW, CHEN YM, LIN CC *et al*.: Elevated expression of the NLRP3 inflammasome and its correlation with disease activity in adult-onset Still disease. *J Rheumatol* 2017; 44(8): 1142-50. https://doi.org/10.3899/jrheum.161354
45. NAGAI H, KIRINO Y, NAKANO H *et al*.: Elevated serum gasdermin D N-terminal implicates monocyte and macrophage pyroptosis in adult-onset Still's disease. *Rheumatology* (Oxford) 2021; 60: 3888-95. https://doi.org/10.1093/rheumatology/keaa814
46. RIVA G, NASILLO V, LUPPI M, TAGLIAFICO E, TRENTI T: Linking COVID-19, monocyte activation and sepsis: MDW, a novel biomarker from cytometry. *EBioMedicine* 2022; 75: 103754. https://doi.org/10.1016/j.ebiom.2021.103754