IM - ORIGINAL

Clinical and laboratory features associated with macrophage activation syndrome in Still's disease: data from the international AIDA Network Still's Disease Registry

Paola Triggianese' · Antonio Vitale"¹ · Giuseppe Lopalco · Henrique Ayres Mayrink Giardini · Francesco Ciccia · Ibrahim Al-Maghlouth', et al. *[full author details at the end of the article]*

Abstract

To characterize clinical and laboratory signs of patients with Still's disease experiencing macrophage activation syndrome (MAS) and identify factors associated with MAS development. Patients with Still's disease classified according to internationally accepted criteria were enrolled in the AutoInflammatory Disease Alliance (AIDA) Still's Disease Registry. Clinical and laboratory features observed during the inflammatory attack complicated by MAS were included in univariate and multivariate logistic regression analysis to identify factors associated to MAS development. A total of 414 patients with Still's disease were included; 39 (9.4%) of them developed MAS during clinical history. At univariate analyses, the following variables were significantly associated with MAS: classification of arthritis based on the number of joints involved ($p = 0.003$), liver involvement ($p = 0.04$), hepatomegaly ($p = 0.02$), hepatic failure ($p = 0.01$), axillary lymphadenopathy ($p = 0.04$), pneumonia ($p = 0.03$), acute respiratory distress syndrome ($p < 0.001$), platelet abnormalities ($p < 0.001$), high serum ferritin levels ($p = 0.009$), abnormal liver function tests ($p = 0.009$), hypoalbuminemia ($p = 0.002$), increased LDH ($p = 0.001$), and LDH serum levels ($p < 0.001$). At multivariate analysis, hepatomegaly (OR 8.7, 95% CI 1.9–52.6, $p = 0.007$) and monoarthritis (OR 15.8, 95% CI 2.9–97.1, $p = 0.001$), were directly associated with MAS, while the decade of life at Still's disease onset (OR 0.6, 95% CI 0.4–0.9, $p = 0.045$), a normal platelet count (OR 0.1, 95% CI 0.01–0.8, $p = 0.034$) or thrombocytosis (OR 0.01, 95% CI 0.0–0.2, $p = 0.008$) resulted to be protective. Clinical and laboratory factors associated with MAS development have been identified in a large cohort of patients based on real-life data.

Keywords Arthritis · Autoinflammatory diseases · Diagnosis · MAS · Prognosis

Introduction

Still's disease is a rare systemic autoinflammatory polygenic disorder capable of affecting both adults (adult-onset Still's disease, AOSD) and pediatric patients (systemic juvenile idiopathic arthritis, sJIA) [1, 2]. Noteworthy, increasing evidence suggests that AOSD and sJIA represent a pathological *continuum* rather than different clinical entities [3]. Still's disease recognizes a wide spectrum of non-specific symptoms and clinical manifestations including fever, arthralgia (with or without arthritis), skin rash (especially salmon-pink rash), pharyngodynia, lymphadenopathy, hepato-splenomegaly, and serositis [4, 5]. Neurological, renal, and ophthalmological involvement can rarely occur, thus worsening clinical outcome and complicating the treatment approach [6]. Common laboratory findings include neutrophilic leukocytosis, elevated C-reactive protein (CRP), increased liver enzymes, and high ferritin levels with low glycosylated ferritin ($\leq 20\%$) [7]. Clinical disease course includes three significant patterns with a different distribution and prognosis, consisting of a self-limited or monophasic pattern, a polycyclic intermittent disease, and a chronic-articular course [8, 9]. However, more recently, a dichotomous pattern with a predominant systemic disease and a predominant articular course has been proposed, with the former characterized by higher body temperature, ferritin serum levels, liver enzymes and thrombocytosis and the latter characterized by a more severe articular involvement [10–12].

Paola Triggianese and Antonio Vitale have contributed equally to this work.

The diagnosis of AOSD is essentially clinical and the accurate exclusion of mimickers such as malignancies, infections, and other autoimmune diseases is necessary to confirm the diagnosis [13]. Classification criteria may be useful in recognizing Still's disease, with Yamaguchi's criteria and Fautrel's criteria being the most frequently employed in adults and the International League of Associations for Rheumatology (ILAR) criteria and/or the Pediatric Rheumatology INternational Trials Organization (PRINTO) criteria used in the pediatric setting [14–17].

Hyperactivation of monocytes/macrophages might play central role in the development of macrophage activation syndrome (MAS), one of the most severe and life-threating complications of Still's disease [8, 9]. This complication has been described in up to 15% of patients with this disease; the mortality rate ranges between 2.3 and 16% in such cases [18]. MAS is a secondary hemophagocytic lymphohistiocytosis (HLH) associated with autoimmune diseases [19]. As described by different authors, HLH and MAS are not discrete diseases, but they are the *continuum* of hemophagocytic disorders sharing common pathways of impaired cytotoxicity leading to a "cytokine inflammatory storm" [1, 20, 21].

The classification criteria more frequently used for MAS in Still's disease have been validated for patients with sJIA [22]. However, they are commonly used also for other systemic autoinflammatory diseases. According to this set of criteria, MAS classification may occur when a febrile patient with known or suspected Still's disease shows high serum ferritin (> 684 ng/ml) and at least two items among decreased platelet count (< 181 × 10^9/l), decreased fibrinogen serum levels (< 360 mg/dl), increased aspartate aminotransferase (AST, > 48 U/l), and increased triglycerides (> 156 mg/dl) [22]. A second score, defined HScore, has been developed and validated for the diagnosis of reactive HLH, both in rheumatologic and non-rheumatologic conditions [23]. It is more frequently used in adult patients. A further set of criteria developed on behalf of the Histiocytic Society (HLH-2004) is also used, but it is primarily intended for genetic forms of HLH [24].

MAS can occur either at the time of diagnosis or later during patients' clinical history, but specific defined predictive factors are lacking or require to be confirmed on large population studies [19]. Similarly, clinical and laboratory clues capable of simplifying identification of MAS would also be needed to improve patients' outcome. On this basis, the aim of the present study is to characterize clinical and laboratory signs of patients with Still's disease experiencing MAS and to identify factors associated with MAS development.

Methods

This cohort study was based on data collected in the International AutoInflammatory Disease Alliance (AIDA) Registry dedicated to Still's disease [25]. Patients with Still's disease, classified according to internationally accepted criteria (Yamaguchi and/or Fautrel and/or ILAR and/or PRINTO criteria [14–17]) were enrolled between 2021 June and 2023 January.

The developing of MAS was defined according to the fulfilment of 2016 MAS criteria and/or the HLH-2004 criteria and/or on the basis of an HScore > 250 (MAS probability > 90%); patients never developing MAS accounted for the comparison group [22–24].

Demographic data included sex, ethnicity, age at the Still's disease onset, diagnostic delay, and Still's disease duration. Disease course was defined as monocyclic, polycyclic, and chronic-articular course [26]; the disease course remained unknown in patients in whom the course was already undefined at the last follow-up. Patients were also stratified in two subgroups based on the age at the Still's disease onset: patients with age at onset < 16 years old were classified as pediatric.

Laboratory variables considered in the analysis were: erythrocyte sedimentation rate (ESR), CRP, aspartate aminotransferase (AST), alanine aminotransferase (ALT), β2-microglobulin, lactate dehydrogenase (LDH), direct and total bilirubin, gamma-glutamyltransferase (GGT), alkaline phosphatase (AP). These variables were recorded either during the attack leading to MAS development or at the worst episode of Still's disease (based on the occurrence or non-occurrence of MAS); they were considered increased in accordance with each laboratory reference limit.

Hypoalbuminemia was defined as < 3.5 g/dl; hypergammaglobulinemia (polyclonal gammopathy) was identified as a broad-based peak or band in the gamma region on serum protein electrophoresis. Anemia was defined as Hb ≤ 12 g/dl; platelets count ≥ 450,000/mm^3 defined thrombocytosis in adults while ≥ 500,000/mm^3 defined thrombocytosis in childhood [27]; platelets count ≤ 150,000/mm^3 defined thrombocytopenia [28]. Abnormal liver function was defined as the occurrence of increased levels of AST and/or ALT and/or direct/total bilirubin and/or GGT, and/or AP, while liver involvement consisted of having abnormal liver function and/or hepatomegaly (identified by ultrasound and/or radiological documentations). Referring to the classification criteria proposed by Yamaguchi et al. [14], hyperferritinemia was defined as a serum ferritin level higher than 3000 ng/ml and leukocytosis as a white blood count exceeding 15,000/mm^3.

Diagnosis of pleuritis and/or pneumonia, pericarditis, and peritonitis was based on ultrasound and/or radiological

documentations; similarly, lymphadenopathy and spleno-megaly were confirmed by ultrasound and/or computed tomography scans.

The occurrence of the following clinical variables refer-ring to the attack leading to MAS development was also registered: highest body temperature (°C), skin rash, thoracic and/or abdominal pain, arthralgia, myalgia, conjunctivitis, kidney involvement, orchitis, neurologic involvement, and severe complications including fulminant hepatic failure and acute respiratory distress syndrome (ARDS). Arthritis was classified as monoarticular (1 joint), oligoarticular (2–4 joints), and polyarticular ($\geq$ 5 joints. Still's disease severity was assessed according to the systemic scores proposed by Pouchot et al. [29] and Rau et al. [30].

Statistical analysis

Continuous variables were described as mean and standard deviation (SD) or as median and interquartile range (IQR), where appropriate. The Kolmogorov–Smirnov test was used to determine normality. Categorical variables were reported as frequencies and percentages. Differences in variables were compared using the unpaired *t* test for normally dis-tributed quantitative data, and the Mann–Whitney test for non-normally distributed quantitative data. The Chi-squared test or the Fisher's exact test were used to analyze associa-tion between categorical variables. Univariate and multi-variate logistic regression analyses were used to identify factors associated with MAS. Univariate logistic regression analyses were performed to assess the unadjusted associa-tion between MAS and clinical/laboratory parameters while stepwise multivariate logistic regression analyses were used to identify independent factors associated with MAS. The

variables entering the multivariate model were statistically significant and clinically relevant at univariate analyses ($p < 0.05$). Only the patients characterized by a good retro-spective data collection (80% of data correctly inserted into the AIDA registry) were included in the univariate regres-sion analysis and in the subsequent multivariate model. Supplementary table 1 provides demographic, clinical and laboratory data accounting for independent variables at univariate and multivariate logistic regression; the MAS development (yes/no) represented the dependent variable. The level of significance was 0.05. All statistical analyses were conducted using STATA 17/MP2 (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC).

Results

We included 414 patients. Ethnicity was as follows: cauca-sic ($n = 344$, 83.1%), Arab ($n = 39$, 9.4%), Hispanic ($n = 18$, 4.3%), Africans ($n = 7$, 1.7%), indigenous peoples of South America ($n = 3$, 0.7%), Asian ($n = 2$, 0.5%), indigenous peo-ples of North America ($n = 1$, 0.24%). Women represented 59.9% of the study population ($n = 248$) (Table 1).

In the whole cohort, the median disease duration at enroll-ment was 0.2 year (IQR 0.8, range 0–22). The mean age at the Still's disease onset was 32.54 ± 17.14 years (range 0.8–79.7) with a median diagnostic delay of 0.2 years (IQR 0.8, range 0–22.9) and a female to male (F:M) ratio of 1.47. Patients had mainly a monocyclic disease course ($n = 129$, 31.2%) followed by a polycyclic course ($n = 104$, 25.1%) and then chronic-articular courses ($n = 86$, 20.8%). However,

Table 1 Demographic data from the study population and disease course

	Study popula-tion, $N = 414$	MAS, $N = 39$	Non-MAS, $N = 375$	*p*
Age at Still's disease onset, yrs, Mean $\pm$ SD	32.54 ± 17.14	25.26 ± 16.49	33.25 ± 16.75	0.003
Diagnostic delay, yrs Median (IQ; range)	0.2 (0.8; 0–22.9)	0.2 (0.9; 0–21.9)	0.1 (0.2; 0–20.5)	0.101
Still's disease duration, yrs Median (IQ; range)	0.2 (0.8; 0–22)	0.1 (0.2; 0–20.4)	0.2 (0.9; 0–22)	0.07
Females (*N/%*)	248/59.9	25/64.1	223/59.5	0.61
Pediatric age onset (*N/%*)	63/15.2	11/28.2	52/13.9	0.02
Monocyclic course (*N/%*)	129/31.2	15/38.5	114/30.4	0.3
Polycyclic course (*N/%*)	104/25.1	13/33.3	91/24.3	0.22
Systemic course (*N/%*)	233/56.3	28/71.8	205/54.7	0.04
Chronic-articular course (*N/%*)	86/20.8	3/7.7	83/22.1	0.04
Unknown course (*N/%*)	95/22.9	8/20.5	87/23.2	0.7

p values refer to comparisons between MAS and non-MAS groups; they were obtained with Mann–Whit-ney *U* test for quantitative data and Chi-square test for qualitative data

IQ interquartile range, *MAS* macrophage activation syndrome, *SD* standard deviation, *yrs* years

the disease course remained still unknown in 22.9% of the cohort ($n = 95$) owing to the short follow up period.

Patients with age at the Still's disease onset < 16 years old represented 15.2% of the study population ($n = 63$, mean age at diagnosis 10.25 ± 2 years). A similar sex distribution occurred in pediatric patients (F:M ratio 1.1), while patients with age at the disease onset ≥ 16 years old ($n = 353$, mean age at diagnosis 38.17 ± 15 yrs) showed a slightly higher female prevalence (F:M ratio 1.55).

Both in pediatric and adult patients, comparable prevalence occurred for the monocyclic (28.6% *vs* 31.6%, respectively, $p = 0.63$) and polycyclic (27% vs 24.2%, respectively, $p = 0.53$) disease course; conversely, the chronic-articular type was significantly less frequent in the pediatric context (30.2% in adults *vs* 19.1% among children, $p = 0.046$). An unknown Still's disease course was registered in 14.3% of pediatric-onset cases and 24.2% of adult-onset cases, $p = 0.08$.

MAS patients

Patients with MAS represented 9.4% ($n = 39$) of the whole cohort, with a F:M ratio of 1.8 (Table 1). Thirty-six (92.3%) patients fulfilled 2016 criteria for MAS [22]; 3 patients fulfilled the HLH-2004 criteria and/or the HScore criteria [23, 24]. Thirty-six (92.3%) patients developed MAS at the start of Still's disease; the remaining 3 (7.7%) patients developed MAS during a subsequent Still's disease flare. Most of MAS patients were Caucasics ($n = 35$, 89.7%); 2 Arabs (5.1%), 1 (2.5%) Hispanic, and 1 (2.5%) indigenous from North America were also observed. The age at Still's disease onset was 25.26 ± 16.49 years, with a median diagnostic delay of 0.2 yr (IQR 0.9, range 0–21.9). None of them died.

Patients with age at the disease onset < 16 y.o. represented 28.2% of the MAS group, with a F:M ratio of 1.5, and a median age at the disease onset of 10.9 years (IQR 9, range 0.8–16 years); patients with age at the disease onset ≥ 16 y.o. showed a F:M ratio of 1.8, and a median age at the disease onset of 30.65 years (IQR 22.1, range 17.3–75.5 years).

Non-MAS patients

Patients with non-MAS were the largest part of the cohort ($n = 375$, 90.6%) with a F:M ratio of 1.5 (Table 1). Caucasic ethnicity included 82.4% of this subgroup ($n = 309$). The age at the disease onset was 33.25 ± 16.75 years, with a median diagnostic delay of 0.1 year (IQR 0.2, range 0–20.5).

Patients with age at the disease onset < 16 y.o. represented 13.9% of the group with a F:M ratio of 0.96, and a median age at the disease onset of 9.9 years (IQR 8.1, range 0.8–15.9 years); patients with age at the disease onset ≥ 16 y.o. showed a F:M ratio of 1.6, and a median age at the disease onset of 34.9 years (IQR 20.8, range 16.1–79.7 years).

Comparing MAS and non-MAS patients

Clinical features

The Still's disease onset at age < 16 y.o. resulted significantly more frequent in the MAS group compared with the non-MAS group ($p = 0.02$). Accordingly, the age at onset resulted lower in MAS than in non-MAS patients ($p = 0.003$) (Table 1).

A chronic-articular disease course was significantly more prevalent in non-MAS group compared with the MAS group ($p = 0.04$, Table 1). However, in MAS group, a slightly higher prevalence occurred for a monocyclic disease course while the polycyclic course was registered in a similar percentage between MAS and non-MAS groups (Table 1).

At univariate analyses, performed on patients with complete data insertion (39 patients with MAS and 330 patients with no MAS history), the following clinical manifestations were significantly associated with MAS: liver involvement ($p = 0.04$), hepatomegaly ($p = 0.02$), hepatic failure ($p = 0.01$), and the development of axillary lymphadenopathy ($p = 0.04$). In addition, the development of pneumonia ($p = 0.03$) and ARDS ($p < 0.001$) were significantly associated with MAS. Similarly, the identification of scleritis during the inflammatory attach was found significantly associated with MAS development ($p = 0.003$). The mean value of the highest body temperature reached during attacks was higher in MAS than in non-MAS patients ($p = 0.03$).

Abdominal pain showed a trend toward a higher frequency in MAS than in non-MAS patients without a statistically significant difference (see Table 2).

The classification of arthritis based on the number of joints involved (monoarthritis/oligoarthritis/polyarthritis) was significantly associated with MAS ($p = 0.003$). In particular, considering all patients with arthritis, the monoarticular subtype was more present in the MAS group than in non-MAS group (4/19, 21% vs 8/188, 4.3%, $p = 0.005$); 2 out of the 4 patients with MAS and monoarthritis were pediatric patients. Joints involved in these cases were the knee ($n = 3$) and the wrist ($n = 1$). Polyarthritis was more prevalent in non-MAS than in MAS (106/188, 56.4% vs 6/19, 31.6%, $p = 0.04$). The oligoarthritic subtype recognized a similar distribution between the two groups (9/19, 47.4% in the MAS group vs 74/188, 39.4% in the non-MAS group, $p = 0.49$) (Fig. 1).

Although the occurrence of serositis (pleuritis and/or pericarditis, and/or peritonitis) showed a similar prevalence in MAS and non-MAS subjects, pleuritis resulted twofold more frequent than pericarditis in MAS patients; conversely, pleuritis and pericarditis showed a comparable occurrence in non-MAS patients (see Table 2).

Table 2 Clinical findings from the study groups

Clinical features	MAS, $N=39$	Non-MAS, $N=375$	p value
Skin rash, N (%)	25 (64.1)	230 (61.3)	0.74
Splenomegaly, N (%)	11 (28.2)	126 (33.6)	0.49
Liver involvement, N (%)	19 (48.7)	123 (32.8)	0.047
Hepatomegaly, N (%)	16 (41)	86 (22.9)	0.02
Lymphadenopathy, N (%)	25 (64.1)	182 (48.5)	0.065
Pneumonia, N (%)	4 (10.3)	17 (4.5)	0.12
Serositis (any site), N (%)	13 (33.3)	102 (27.2)	0.42
Pleuritis, N (%)	8 (20.5)	50 (13.3)	0.22
Pericarditis, N (%)	4 (10.3)	50 (13.3)	0.59
Peritonitis, N (%)	1 (2.6)	3 (0.8)	0.28
Thoracic pain, N (%)	4 (10.3)	37 (9.9)	0.94
Abdominal pain, N (%)	10 (25.6)	46 (12.3)	0.06
Arthritis, N (%)	21 (53.8)	216 (57.6)	0.65
Myalgia, N (%)	23 (59)	186 (49.6)	0.27
Eye involvement, N (%)	5 (12.8)	15 (4)	0.02
Scleritis, N (%)	3 (7.7)	0 (0)	<0.001
Conjunctivitis, N (%)	2 (5.1)	11 (3)	0.46
Kidney, N (%)	2 (5.1)	7 (1.9)	0.18
Orchitis, N (%)	0/0 (0)	3 (0.8)	0.58
Neurologic, N (%)	2 (5.1)	9 (2.4)	0.31
ARDS, N (%)	4 (10.3)	1 (0.3)	<0.001
Fulminant hepatic failure, N (%)	2 (5.1)	1 (0.3)	0.001
Highest BT, °C; median (IQR)	40 (0.65)	39.5 (1.0)	0.02
Highest BT ≥ 39 °C, N (%)	38 (97.4)	317 (84.5)	0.03

p values were obtained with Student's t test for quantitative data and Chi-square test or Fisher exact test (according to the sample size and expected frequencies) for qualitative data

ARDS acute respiratory distress syndrome, *BT* body temperature, *IQR* interquartile range, *MAS* macrophage activation syndrome
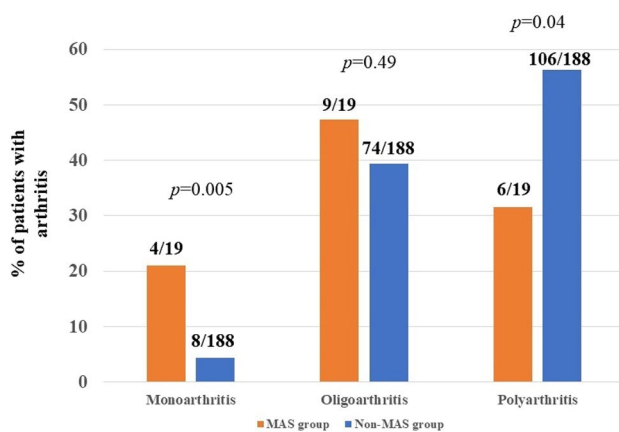

Fig. 1 Types of arthritis in Still's patients according to the number of joints involved. Patients with macrophage activation syndrome (MAS) showed significantly different distributions in the number of joints involved by arthritis, when compared to patients without MAS. p values were obtained with the Chi-squared test or the Fisher's exact test were according to frequency counts and expected frequencies

The prevalence of other clinical Still's disease related manifestation was similar in the two study groups as described in Table 2.

Laboratory findings

At univariate analysis, the following laboratory parameter results were significantly associated with MAS: platelet abnormalities ($p < 0.001$), high serum ferritin levels ($p = 0.003$), abnormal liver function tests ($p = 0.009$)—especially AST ($p = 0.002$), ALT ($p = 0.04$), total bilirubin ($p = 0.049$), direct bilirubin ($p = 0.004$)—and hypoalbuminemia ($p = 0.002$) (Table 3). Moreover, patients with increased LDH were prevalent in MAS group than in non-MAS ($p = 0.001$), while LDH serum levels were higher among MAS patients than in non-MAS patients ($p < 0.001$) (Table 3).

Levels of ESR and CRP were not found to discriminate MAS and non-MAS groups in a significant fashion; similarly, anemia, leukocytosis, the white blood cells count, and

Table 3 Laboratory findings from the study groups

Laboratory findings	MAS, N = 39	Non-MAS, N = 375	p value
ESR, mm/h; mean ± SD	75.5 ± 29.4	79.5 ± 32.4	0.43
CRP mg/dl; median (IQR)	16 (20.5)	13.35 (23.175)	0.21
Anemia, N (%)	23 (58.9)	204 (54.4)	0.69
Leukocytosis, N (%)	29 (74.4)	255 (68)	0.58
White blood cell count, cells/mmc; median (IQR)	18,000 (4150)	16,955 (5400)	0.15
Absolute neutrophil count, cells/mmc; median (IQR)	15,662 (5320)	14,434.5 (5782)	0.06
Thrombocytosis, N (%)	3 (9.4)	83 (22.1)	0.04
Normal platelet count, N (%)	20 (51.3)	270 (72)	0.03
Thrombocytopenia, N (%)	16 (41)	22 (5.9)	< 0.001
Abnormal liver function, N (%)	26 (66.7)	153 (40.8)	0.009
Increased AST, N (%)	25 (64.1)	119 (31.7)	0.002
Increased ALT, N (%)	22 (56.4)	120 (32)	0.04
Conjugated hyperbilirubinemia, N (%)	5 (12.8)	6 (1.6)	0.004
Total hyperbilirubinemia, N (%)	4 (10.3)	6 (1.6)	0.049
Hypoalbuminemia, N (%)	21 (53.8)	90 (24)	0.002
Increased ferritin, N (%)	36 (92.3)	318 (84.8)	0.18
Ferritin, ng/ml; median (IQR)	6908 (30,106.5)	1559.5 (5355)	0.003
Increased LDH, N (%)	29 (74.4)	146 (38.9)	0.001
LDH (U/L, mean ± SD)	790 (1906.5)	487 (219)	< 0.001
Hypergammaglobulinemia, N (%)	8 (20.5)	63 (16.8)	0.99
Increased β2-microglobulin, N (%)	5 (12.8)	80 (21.3)	0.65

p values were obtained with Student's t test or Mann–Whitney U test (according to data distribution) for quantitative data and Chi-square test for qualitative data

ALT alanine aminotransferase, AST aspartate aminotransferase, CRP C-reactive protein, ESR erythrocyte sedimentation rate, IQR interquartile range, LDH lactate dehydrogenase, MAS macrophage activation syndrome, SD standard deviation

the absolute neutrophil count were comparable between the two groups (Table 3).

Multivariate analysis

After the stepwise procedure, the variables that resulted statistically significant were hepatomegaly (OR 8.7, 95% CI 1.9–52.6, $p = 0.007$) and monoarthritis (OR: 15.8, 95% CI 2.9–97.1, $p = 0.001$), that were directly associated with MAS development. The age at Still's disease onset (OR 0.6, 95% CI 0.4–0.9, $p = 0.045$, the OR indicates the risk for a 10-year increment of age), a normal platelet count (OR 0.1, 95% CI 0.01–0.8, $p = 0.034$) and the presence of thrombocytosis (OR 0.02, 95% CI 0.0–0.2, $p = 0.008$) were inversely associated with MAS development (Fig. 2).

Clinical severity scores

The median value of the systemic score for Still's disease severity was 6 (IQR = 3) in the MAS group and 6 (IQR = 2) in the non-MAS group according to Pouchot et al.; it was significantly higher in the MAS group ($p = 0.01$). The same result occurred by analyzing clinical manifestations using the systemic score modified by Rau et al., whose median

value resulted to be 6 (3) in the MAS group and 5 (3) in the non-MAS group; it was significantly higher in the former group ($p = 0.004$) (Fig. 3). A systemic Pouchot score ≥ 7 was significantly more frequent among MAS patients than in non-MAS patients (12/39 versus 60/357 patients, $p = 0.02$); conversely, the systemic score by Rau et al. was not significantly different among groups (13/39 versus 82/357, $p = 0.11$).

Discussion

The lack of clinical and reliable clues to early and effective detection of MAS often concur to the inadequate treatment and to the poor outcome for this disorder [31, 32]. We suggest here defined clinical indicators capable of associating with MAS development in Still's disease and useful at improving the early detection of this life-threatening complication. In particular, the number of joints involved by arthritis, the occurrence of liver involvement, pneumonia, ARDS, and axillary lymphadenopathy appear associated with MAS development, along with the identification of thrombocytopenia, hyperferritinemia, hypoalbuminemia, increased LDH, and abnormal liver function tests.
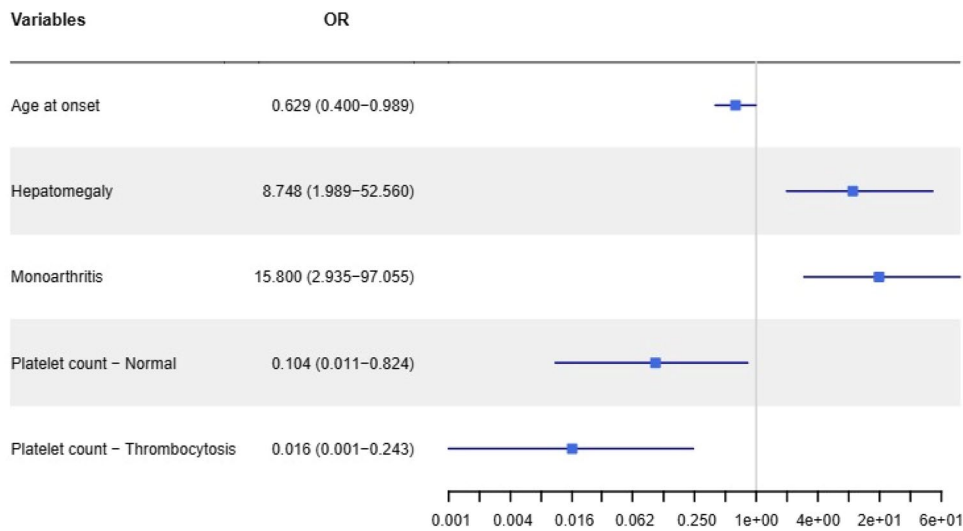
Variables	OR
Age at onset	0.629 (0.400–0.989)
Hepatomegaly	8.748 (1.989–52.560)
Monoarthritis	15.800 (2.935–97.055)
Platelet count – Normal	0.104 (0.011–0.824)
Platelet count – Thrombocytosis	0.016 (0.001–0.243)

Fig. 2 Results of multivariate logistic regression analysis. Odds Ratios and 95% confidence interval of variables significantly associated with MAS development; x-axis was represented with logarithmic scale to facilitate graphical description. As noted, patients with a normal platelet count or even more with thrombocytosis have a lower probability to develop MAS; similarly, this probability decreases along with the increase of the age at the time of disease onset, provided as decades of life at the time of disease onset. On the contrary, patients with monoarthritis and hepatomegaly are more prone to develop MAS. The figure represents the outcome of a stepwise multivariate logistic regression analysis. Forty non-MAS patients characterized by an inadequate retrospective data collection (less than 80% of data required) were not considered for regression analysis
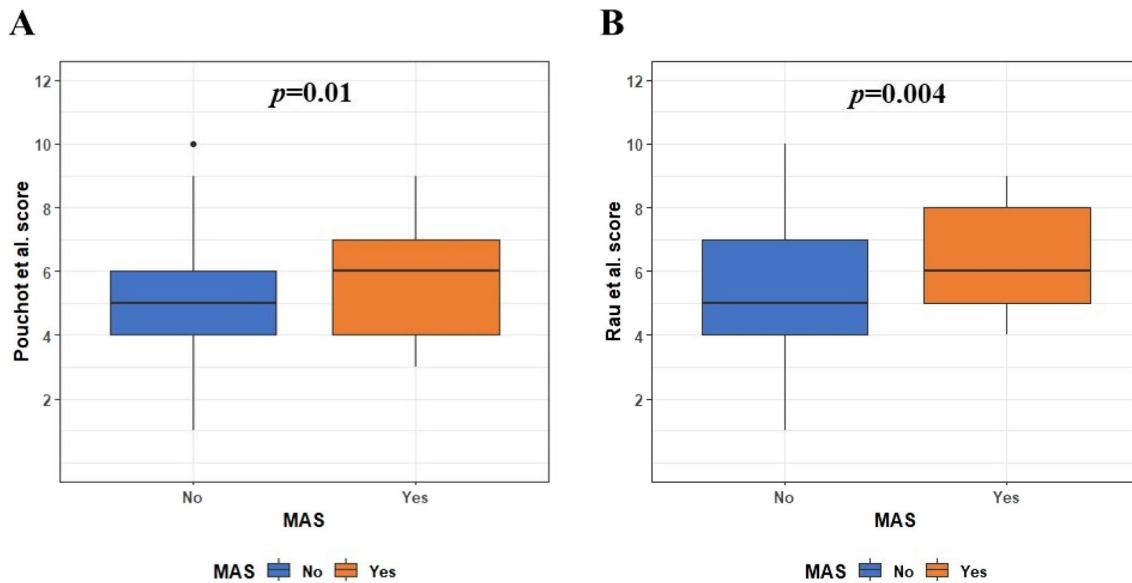
Fig. 3 Systemic scores for Still's disease severity. Patients with macrophage activation syndrome (MAS) reported significantly higher Pouchot et al. score [29] and Rau et al. score [30], compared to the non-MAS group, as represented in **A** and **B**, respectively. p values were obtained using the Mann–Whitney test for non-normally distributed continuous data. The lower whiskers correspond to 1.5 times the first quartile; the upper whiskers correspond to 1.5 times the third quartile

In particular, the presence of hepatomegaly and monoarthritis have shown to be associated with the development of MAS. The association with monoarthritis confirms the higher frequency of MAS among patients with the systemic course rather than among subjects with the chronic-articular disease, which is typically characterized by a more prominent articular involvement.

Patients with a normal platelet count or even more with thrombocytosis have a lower probability to develop MAS, as observed at multivariate analysis. Similarly, an older age

at disease onset results protective for MAS, with a reduction of the risk as the patients' age increases at the start of symptoms. To further support this finding, a significantly higher prevalence of pediatric patients results in the MAS group compared with the non-MAS group, while age at onset was lower in MAS than in non-MAS patients. These results are clinically relevant, as the age at Still's disease onset may occur at any age and the risk for MAS development reduces as the decades of life increase at the onset of Still's disease.

The lower prevalence of the chronic-articular disease course in MAS patients reflects the well-known features of the systemic type, which is characterized by a more pronounced cytokine storm-induced inflammation [33]. Consequently, our findings confirm that MAS arises more frequently in patients with a more prominent inflammatory phenotype. Polyarthritis was more prevalent in Still's disease patients who do not develop MAS. This is in accordance with the lower frequency of MAS among patients with the chronic-articular disease course and the lower inflammatory burden observed in this type of disease [33, 34]. This also corroborates the protective role of increasing age against MAS development, as the chronic-articular Still's disease course was significantly more frequent among patients with adult disease onset.

At univariate analyses, the present study defines liver involvement and the occurrence of axillary lymphadenopathy as clinical manifestations significantly associated with MAS development. These clues agree with findings observed in previous studies [35, 36]. In particular, strong associations have already been found between liver involvement and ferritin serum levels, which may act as a pathogenic protein contributing to the development of a self-perpetuating cytokine storm [37, 38]. Therefore, patients with liver involvement might indicate exaggerated inflammatory responses in Still's disease patients [39]. Previous studies also described that Still's disease patients with MAS are more likely to have hepatomegaly than Still's disease without MAS and that patients with liver involvement have an almost sixfold higher risk of MAS [35, 40]. Noteworthy, MAS as HLH can present with wide range of hepatic dysfunction ranging from mild abnormalities of transaminases to liver failure. Particularly, MAS patients with liver failure might experience ARDS and/or respiratory failure due to pneumonia [41, 42]. In this regard, lung involvement has been recognized as an emergent cause of mortality and a marker of poor prognosis in Still's disease [43]; also, it has been found associated with MAS and seems to define a distinct clinical and immunologic pattern in such cases [44]. The present work corroborates all these observations.

Pleuritis and pericarditis represent relevant features for Still's disease [45]. Our data show that pleuritis is twofold frequent than pericarditis in Still's disease patients developing MAS. This is in agreement with previously reported evidence supporting that Still's disease patients with pleuritis also experience a higher frequency of disseminated intravascular coagulation and MAS [46]. Furthermore, patients with Still's disease and serosal involvement have proved to be more likely to develop MAS [36, 47].

Concerning laboratory investigations, our results document that thrombocytopenia, elevated serum ferritin, LDH levels, abnormal liver function—in particular AST, ALT, total bilirubin, direct bilirubin, and hypoalbuminemia—are significantly associated with MAS. Moreover, patients with increased LDH are more prevalent in the MAS group than in non-MAS group. These findings support previous data about the role of laboratory investigations in identifying patients with MAS. In particular, dynamic changes in some laboratory data, especially platelet count, were found to differentiate MAS from flare-ups of autoimmune diseases, while changes in liver enzymes levels, high ferritin levels and white blood cells may enhance an early diagnosis of MAS [48, 49]. Accordingly, a recent study aimed at identifying valuable serum laboratory markers reflecting MAS disease activity documented that significant changes in platelet count, LDH levels, and D-dimers accounted for the most valuable indicators of MAS in patients with systemic JIA [50]. Other authors documented that extremely elevated serum LDH levels represented useful diagnostic markers for MAS along with moderate to severe lymphopenia [51]. Similarly, the levels of AST, LDH, and platelet count resulted to be associated with a poor prognosis in patients with rheumatic diseases and MAS, including those with Still's disease [52].

Both the systemic Pouchot score [29] and the modified systemic score by Rau et al. [30], used to assess disease severity, have proved to be significantly higher in the MAS group compared with non-MAS patients. In this regard, the systemic score values by Pouchot et al. had already been found to be significantly higher in patients developing MAS [35]. The present study confirms the usefulness of the systemic score by Pouchot et al. as clinical tool to be used in patients with suspected MAS. In addition, our results indicate that the systemic score modified by Rau et al. may have a similar role in warning physicians to the possible development of MAS. Noteworthy, a Pouchot score higher than 7 was significantly more frequent in the MAS group than in non-MAS patients, confirming the capability of this cut-off in recognizing patients at higher risk of death [53].

Limitations of the study include the retrospective design and the relatively small number of patients developing MAS. Diagnostic criteria for MAS in Still's disease used in this study have not been validated for adult patients with Still's disease [22]. Nevertheless, they are generally used for patients with autoinflammatory diseases and suspected MAS. Also, data from pediatric patients were meshed with those referring to adult patients. In this regard, the age at disease onset appears to be a variable associated with MAS

development rather than a confounding factor, and this was strongly highlighted by combining pediatric-onset patients with adults.

Of note, the lack of deaths could be related to a better management and an earliest recognition of MAS in recent times compared with what recorded in previous decades [18]; however, it could also conceal a selection bias, as investigators may have omitted the recruitment of deceased patients. If so, the power of factors identified in this study in recognizing the most severe MAS patients could be partially affected and future studies should evaluate whether the results achieved in this study are superimposed on those that will be obtained from patients with fatal MAS. However, based on real-life data reflecting the daily clinical practice, this study largely confirms the findings previously obtained from smaller cohorts of patients. It thus provides the clinical and laboratory clues to take into account when dealing with Still's disease patients suspected to complicate with MAS.

In conclusion, the risk of developing MAS is higher among pediatric patients. Subjects with systemic disease course, monoarthritis, liver involvement, pneumonia, ARDS and axillary lymphadenopathy are more likely to develop MAS; this probability is lower among patients with polyarthritis and chronic-articular disease course. Thrombocytopenia, abnormalities in liver function tests, serum ferritin levels, LDH, and hypoalbuminemia represent laboratory findings to be primarily considered for early detection of patients developing MAS.

Declarations

References

1. Ruscitti P, Giacomelli R (2018) Pathogenesis of adult onset Still's disease: current understanding and new insights. Expert Rev Clin Immunol 14:965–976
2. Sfriso P, Priori R, Valesini G, Rossi S, Montecucco CM, D'Ascanio A et al (2016) Adult-onset Still's disease: an Italian multicentre retrospective observational study of manifestations and treatments in 245 patients. Clin Rheumatol 35:1683–1689
3. Inoue N, Shimizu M, Tsunoda S, Kawano M, Matsumura M, Yachie A (2016) Cytokine profile in adult-onset Still's disease: comparison with systemic juvenile idiopathic arthritis. Clin Immunol 169:8–13
4. Feist E, Mitrovic S, Fautrel F (2018) Mechanisms, biomarkers and targets for adult onset Still's disease. Nat Rev Rheumatol 14:603–618
5. Giacomelli R, Ruscitti P, Shoenfeld Y (2018) A comprehensive review on adult onset Still's disease. J Autoimmun 93:24–36
6. Gerfaud-Valentin M, Jamilloux I, Iwaz J, Sève P (2014) Adult onset Still's disease. Autoimmun Rev 13:708–712
7. Mitrovic S, Fautrel B (2018) New markers for adult-onset Still's disease. Jt Bone Spine 85:285–293
8. Asanuma YF, Mimura T, Tsuboi H, Noma H, Miyoshi F, Yamamoto K et al (2015) Nationwide epidemiological survey of 169 patients with adult Still's disease in Japan. Mod Rheumatol 25:393–400
9. Efthimiou P, Kontzias A, Hur P, Rodha K, Ramakrishna GS, Nakasato P (2021) Adult-onset Still's disease in focus: clinical manifestations, diagnosis, treatment, and unmet needs in the era of targeted therapies. Semin Arthritis Rheum 51:858–874
10. Franchini S, Dagna L, Salvo F, Aiello P, Baldissera E, Sabbadini M (2010) Efficacy of traditional and biologic agents in different clinical phenotypes of adult-onset still's disease. Arthritis Rheumatol 62:2530–2535
11. Jamilloux Y, Gerfaud-Valentin M, Martinon F, Belot A, Henry T, Sève P (2014) Pathogenesis of adult-onset Still's disease: new insights from the juvenile counterpart. Immunol Res 61:53–62
12. Chen D, Hsieh T, Chen Y, Hsieh C, Lan J, Lin F (2008) Proinflammatory cytokine profiles of patients with elderly-onset rheumatoid

arthritis: a comparison with younger-onset disease. Gerontology 55:250–258

13. Lebrun D, Mestrallet S, Dehoux M, Golmard J, Granger B, Georgin-Lavialle S et al (2018) Validation of the Fautrel classification criteria for adult-onset Still's disease. Semin Arthritis Rheum 47:578–585

14. Yamaguchi M, Ohta A, Tsunematsu T, Kasukawa R, Mizushima Y, Kashiwagi H et al (1992) Preliminary criteria for classification of adult Still's disease. J Rheumatol 19:424–430

15. Fautrel B, Zing E, Golmard JL, LeMoel G, Bissery A, Rioux C et al (2000) Proposal for a newest of classification criteria for adult-onset still disease. Medicine (Baltimore) 81:194–200

16. Petty RE, Southwood TR, Manners P, Baum J, Glass DN, Goldenberg J et al (2004) International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol 31:390–392

17. Martini A, Ravelli A, Avcin T, Beresford MW, Burgos-Vargas R, Cuttica R et al (2019) Toward new classification criteria for juvenile idiopathic arthritis: first steps, pediatric rheumatology international trials organization international consensus. J Rheumatol 46:190–197

18. Tomaras S, Goetzke C, Kallinich T, Feist E (2021) Adult-onset Still's disease: clinical aspects and therapeutic approach. J Clin Med 10:733

19. Ravelli A (2002) Macrophage activation syndrome. Curr Opin Rheumatol 14:548–552

20. Halyabar O, Chang MH, Schoettler ML, Schwartz MA, Baris EH, Benson LA et al (2019) Calm in the midst of cytokine storm: a collaborative approach to the diagnosis and treatment of hemophagocytic lymphohistiocytosis and macrophage activation syndrome. Pediatr Rheumatol Online J 17:7

21. Buda P, Strauss E, Januszkiewicz-Lewandowska D, Czerwinska E, Ludwikowska K, Szenborn L et al (2022) Clinical characteristics of children with MIS-C fulfilling classification criteria for macrophage activation syndrome. Front Pediatr 10:981711

22. Ravelli A, Minoia F, Davì S, Horne A, Bovis F, Pistorio A et al (2016) 2016 classification criteria for macrophage activation syndrome complicating systemic juvenile idiopathic arthritis. Ann Rheum Dis 75:481–489

23. Fardet L, Galicier L, Lambotte O, Marzac C, Aumont C, Chahwan D et al (2014) Development and validation of the HScore, a score for the diagnosis of reactive hemophagocytic syndrome. Arthritis Rheumatol 66:2613–2620

24. Henter JI, Horne A, Aricó M, Egeler RM, Filipovich AH, Imashuku S et al (2007) HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 48:124–131

25. Sota J, Vitale A, Lopalco G, Pereira RMR, Giordano HF, Antonelli IPB et al (2022) Efficacy and safety of tocilizumab in adult-onset Still's disease: real-life experience from the international AIDA registry. Semin Arthritis Rheum 57:152089

26. Cush JJ, Medsger TA, Christy WC, Herbert DC, Cooperstein LA (1987) Adult-onset Still's disease. Clinical course and outcome. Arthritis Rheum 30:186–194

27. Sutor AH (1995) Thrombocytosis in childhood. Semin Thromb Hemost 21:330–339

28. Gauer RL, Braun MM (2012) Thrombocytopenia. Am Fam Physician 85:612–622

29. Pouchot J, Sampalis JS, Beaudet F, Carette S, Decary F, Salusinsky-Sternbach M et al (1991) Adult Still's disease: manifestations, disease course, and outcome in 62 patients. Medicine (Baltimore) 70:118–136

30. Rau M, Schiller M, Krienke S, Heyder P, Lorenz HM, Blank N (2010) Clinical manifestations but not cytokine profiles differentiate adult onset Still's disease and sepsis. J Rheumatol 37:2369–2377

31. Ailioaie LM, Ailioaie C, Litscher G (2022) Biomarkers in systemic juvenile idiopathic arthritis, macrophage activation syndrome and their importance in COVID era. Int J Mol Sci 23:12757

32. Naveen R, Guleria S, Mohindra N, Aggarwal A (2023) Predictors of long-term functional outcomes of juvenile idiopathic arthritis-enthesitis related arthritis: a single centre experience. Rheumatology (Oxford) 62:kead032

33. Shimizu M, Nakagishi Y, Yachie A (2013) Distinct subsets of patients with systemic juvenile idiopathic arthritis based on their cytokine profiles. Cytokine 61:345–348

34. Fujii T, Nojima T, Yasuoka H, Satoh S, Nakamura K, Kuwana M et al (2001) Cytokine and immunogenetic profiles in Japanese patients with adult still's disease. Association with chronic articular disease. Rheumatology 40:1398–1404

35. Ruscitti P, Iacono D, Ciccia F, Emmi G, Cipriani P, Grembiale RD et al (2018) 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 45:864–872

36. Yang XP, Wang M, Li TF, Li W, Zhang L, Liu SY (2019) Predictive factors and prognosis of macrophage activation syndrome associated with adult-onset Still's disease. Clin Exp Rheumatol 37(Suppl 121):83–88

37. Ruddell RG, Hoang-Le D, Barwood JM, Rutherford PS, Piva TJ, Watters DJ et al (2009) Ferritin functions as a proinflammatory cytokine via iron-independent protein kinase C zeta/nuclear factor kappaB-regulated signaling in rat hepatic stellate cells. Hepatology 49:887–900

38. Rosario C, Zandman-Goddard G, Meyron-Holtz EG, D'Cruz DP, Shoenfeld Y (2013) The hyperferritinemic syndrome: macrophage activation syndrome, Still's disease, septic shock and catastrophic antiphospholipid syndrome. BMC Med 11:185

39. Chi H, Wang Z, Meng J, Han P, Zhai L, Feng T et al (2020) A cohort study of liver involvement in patients with adult-onset still's disease: prevalence, characteristics and impact on prognosis. Front Med (Lausanne) 7:621005

40. Javaux C, El-Jammal T, Neau PA, Fournier N, Gerfaud-Valentin M, Perard L et al (2021) Detection and prediction of macrophage activation syndrome in Still's disease. J Clin Med 11:206

41. Jagtap N, Sharma M, Rajesh G, Rao PN, Anuradha S, Tandan M et al (2017) Hemophagocytic lymphohistiocytosis masquerading as acute liver failure: a single center experience. J Clin Exp Hepatol 7:184–189

42. Carter SJ, Tattersall RS, Ramanan AV (2019) Macrophage activation syndrome in adults: recent advances in pathophysiology, diagnosis and treatment. Rheumatology (Oxford) 58:05–17

43. Ruscitti P, Berardicurti O, Iacono D, Pantano I, Liakouli V, Caso F et al (2020) Parenchymal lung disease in adult onset Still's disease: an emergent marker of disease severity-characterisation and predictive factors from Gruppo Italiano di Ricerca in Reumatologia Clinica e Sperimentale (GIRRCS) cohort of patients. Arthritis Res Ther 22:151

44. Schulert GS, Yasin S, Carey B, Chalk C, Do T, Schapiro AH et al (2019) Systemic juvenile idiopathic arthritis-associated lung disease: characterization and risk factors. Arthritis Rheumatol 71:1943–1954

45. Mueller RB, Sheriff A (2010) Scoring adult-onset Still's disease. J Rheumatol 37:2203–2204

46. Kishida D, Ichikawa T, Takamatsu R, Nomura S, Matsuda M, Ishii W et al (2022) Clinical characteristics and treatment of elderly onset adult-onset Still's disease. Sci Rep 12:6787

47. Li Y, Guo R, Li W, Feng J, Jin Y, Li J et al (2022) Serosal involvement in adult-onset Still's disease: a multicenter and retrospective study. Mod Rheumatol 33:roac048

48. Assari R, Ziaee V, Mirmohammadsadeghi A, Moradinejad MH (2015) Dynamic changes, cut-off points, sensitivity, and

specificity of laboratory data to differentiate macrophage activation syndrome from active disease. Dis Markers 2015:424381

49. Ruscitti P, Bruno F, Berardicurti O, Acanfora C, Pavlych V, Palumbo P et al (2020) Lung involvement in macrophage activation syndrome and severe COVID-19: results from a cross-sectional study to assess clinical, laboratory and artificial intelligence-radiological differences. Ann Rheum Dis 79:1152–1155

50. Kaneko S, Shimizu M, Miyaoka F, Shimbo A, Irabu H, Mizuta M et al (2023) The dynamics of laboratory markers reflecting cytokine overproduction in macrophage activation syndrome complicated with systemic juvenile idiopathic arthritis. Clin Immunol 248:109270

51. Shen Z, Ling J, Zhu X, Yang J, He T (2023) Macrophage activation syndrome in children with Kikuchi-Fujimoto disease. Pediatr Rheumatol Online J 21:10

52. Nam SH, Ahn SM, Oh JS, Hong S, Lee CK, Yoo B et al (2022) Macrophage activation syndrome in rheumatic disease: clinical characteristics and prognosis of 20 adult patients. PLoS ONE 17:e0267715

53. Ruscitti P, Cipriani P, Masedu F, Iacono D, Ciccia F, Liakouli V et al (2016) 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 14:194

Authors and Affiliations

Paola Triggianese[1,2] · Antonio Vitale[3,4,61] · Giuseppe Lopalco[5] · Henrique Ayres Mayrink Giardini[6] · Francesco Ciccia[7] · Ibrahim Al-Maghlouth[8,9] · Piero Ruscitti[10] · Petros Paul Sfikakis[11] · Florenzo Iannone[5] · Isabele Parente de Brito Antonelli[6] · Martina Patrone[7] · Kazi Nur Asfina[9] · Ilenia Di Cola[10] · Katerina Laskari[11] · Carla Gaggiano[3,4] · Abdurrahman Tufan[12] · Paolo Sfriso[13] · Lorenzo Dagna[14,15] · Roberto Giacomelli[16,17] · Andrea Hinojosa-Azaola[18] · Gaafar Ragab[19,20] · Lampros Fotis[21] · Haner Direskeneli[22] · Veronica Spedicato[5] · Marilia Ambiel Dagostin[6] · Daniela Iacono[7] · Hebatallah Hamed Ali[9] · Paola Cipriani[10] · Jurgen Sota[3,4] · Riza Can Kardas[12] · Sara Bindoli[13] · Corrado Campochiaro[14,15] · Luca Navarini[16,17] · Stefano Gentileschi[3,4] · Eduardo Martín-Nares[18] · Jiram Torres-Ruiz[18] · Moustafa Ali Saad[19] · Katerina Kourtesi[21] · Fatma Alibaz-Oner[22] · Gizem Sevik[22] · Annamaria Iagnocco[23] · Joanna Makowska[24] · Marcello Govoni[25] · Sara Monti[26] · Maria Cristina Maggio[27] · Francesco La Torre[28] · Emanuela Del Giudice[29] · José Hernández-Rodríguez[30] · Elena Bartoloni[31] · Giacomo Emmi[32,33] · Maria Sole Chimenti[1] · Armin Maier[34] · Gabriele Simonini[35] · Giovanni Conti[36] · Alma Nunzia Olivieri[37] · Maria Tarsia[3,4] · Amato De Paulis[38,39] · Alberto Lo Gullo[40] · Ewa Więsik-Szewczyk[41] · Ombretta Viapiana[42] · Benson Ogunjimi[43,44,45,46] · Samar Tharwat[47,48] · Sukran Erten[49] · Rossana Nuzzolese[3,4] · Anastasios Karamanakos[50] · Micol Frassi[51] · Alessandro Conforti[52] · Valeria Caggiano[3,4] · Achille Marino[53] · Gian Domenico Sebastiani[54] · Antonio Gidaro[55] · Enrico Tombetti[56,57] · Francesco Carubbi[58] · Giovanni Rubegni[59] · Alessandra Cartocci[60] · Alberto Balistreri[60] · Claudia Fabiani[59] · Bruno Frediani[3,4] · Luca Cantarini[3,4,61]

✉ Antonio Vitale
vitale.reumatologia@gmail.com

Luca Cantarini
cantariniluca@hotmail.com

1 Rheumatology, Allergology and Clinical Immunology, Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy

2 University of Rome Tor Vergata, Rome, Italy

3 Department of Medical Sciences, Surgery and Neurosciences, Research Center of Systemic Autoinflammatory Diseases, Behçet's Disease Clinic and Rheumatology-Ophthalmology Collaborative Uveitis Center, University of Siena, Siena, Italy

4 UOC Reumatologia, Azienda Ospedaliero-Universitaria Senese, ERN-RITA Center, Siena, Italy

5 Rheumatology Unit, Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy

6 Rheumatology Division, Faculdade de Medicina, Hospital das Clínicas, Universidade de São Paulo, São Paulo, Brazil

7 Department of Precision Medicine, Università Degli Studi Della Campania Luigi Vanvitelli, Naples, Italy

8 Rheumatology Unit, Department of Medicine, College of Medicine, King Saud University, Riyadh, Saudi Arabia

9 College of Medicine Research Center, College of Medicine, King Saud University, Riyadh, Saudi Arabia

10 Rheumatology Unit, Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy

11 Joint Academic Rheumatology Program, National and Kapodistrian University of Athens Medical School, Athens, Greece

12 Division of Rheumatology, Department of Internal Medicine, Gazi University Faculty of Medicine, Ankara, Turkey

13 Rheumatology Unit, Department of Medicine (DIMED), University of Padova, Padua, Italy

14 Unit of Immunology, Rheumatology, Allergy and Rare Diseases, IRCCS San Raffaele Hospital, Milan, Italy

15 Vita-Salute San Raffaele University, Milan, Italy

16 Clinical and Research Section of Rheumatology and Clinical Immunology, Fondazione Policlinico Campus Bio-Medico, Via Álvaro del Portillo 200, 00128 Rome, Italy

17 Rheumatology and Clinical Immunology, Department of Medicine, School of Medicine, University of Rome "Campus Biomedico", Rome, Italy

18 Department of Immunology and Rheumatology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico

19 Internal Medicine Department, Rheumatology and Clinical Immunology Unit, Faculty of Medicine, Cairo University, Giza, Egypt

20 Faculty of Medicine, Newgiza University, 6th of October City, Egypt

21 Third Department of Paediatrics, National and Kapodistrian University of Athens, General University Hospital "Attikon", Athens, Greece

22 Department of Internal Medicine, Division of Rheumatology, School of Medicine, Marmara University, Istanbul, Turkey

23 Academic Rheumatology Center, Dipartimento Scienze Cliniche e Biologiche, Università degli Studi di Torino, Turin, Italy

24 Department of Rheumatology, Medical University of Lodz, Lodz, Poland

25 Rheumatology Unit, Department of Medical Sciences, Azienda Ospedaliero-Universitaria S. Anna-Ferrara, University of Ferrara, Ferrara, Italy

26 Rheumatology Department, Istituto di Ricovero e Cura a Carattere Scientifico Policlinico S. Matteo Fondazione, University of Pavia, Pavia, Italy

27 University Department Pro.Sa.M.I. "G. D'Alessandro", University of Palermo, Palermo, Italy

28 Clinical Pediatrics, University of Bari, Bari, Italy

29 Pediatric and Neonatology Unit, Department of Maternal Infantile and Urological Sciences, Sapienza University of Rome, Polo Pontino, Latina, Italy

30 Vasculitis Research Unit and Autoinflammatory Diseases Clinical Unit, Department of Autoimmune Diseases, Hospital Clinic of Barcelona, IDIBAPS, University of Barcelona, [European Reference Network (ERN) for Rare Immunodeficiency, Autoinflammatory and Autoimmune Diseases (RITA) Center], Barcelona, Spain

31 Department of Medicine and Surgery, MED/16-Rheumatology, Università degli Studi di Perugia, P.Zza Università, 06123 Perugia, Italy

32 Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

33 Centre for Inflammatory Diseases, Department of Medicine, Monash Medical Centre, Monash University, Melbourne, Australia

34 Rheumatology Unit, Department of Medicine, Central Hospital of Bolzano, Bolzano, Italy

35 NEUROFARBA Department, Rheumatology Unit, Meyer Children's University Hospital, University of Florence, Florence, Italy

36 Pediatric Nephrology and Rheumatology Unit, AOU Policlinic G Martino, Messina, Italy

37 Department of Woman, Child and of General and Specialized Surgery, University of Campania "Luigi Vanvitelli", Naples, Italy

38 Section of Clinical Immunology, Department of Translational Medical Sciences, University of Naples Federico II, Naples, Italy

39 Department of Translational Medical Sciences, Center for Basic and Clinical Immunology Research (CISI), World Allergy Organisation Center of Excellence, University of Naples Federico II, Naples, Italy

40 Unit of Rheumatology, Department of Medicine, ARNAS Garibaldi Hospital, Catania, Italy

41 Department of Internal Medicine, Pulmonology, Allergy and Clinical Immunology, Central Clinical Hospital of the Ministry of National Defence, Military Institute of Medicine, National Research Institute, Warsaw, Poland

42 Rheumatology Unit, Department of Medicine, University and Azienda Ospedaliera Universitaria Integrata of Verona, Verona, Italy

43 Antwerp Unit for Data Analysis and Computation in Immunology and Sequencing, University of Antwerp, Antwerp, Belgium

44 Antwerp Center for Translational Immunology and Virology, Vaccine and Infectious Disease Institute, University of Antwerp, Antwerp, Belgium

45 Department of Paediatrics, Antwerp University Hospital, Antwerp, Belgium

46 Center for Health Economics Research and Modeling Infectious Diseases, Vaccine and Infectious Disease Institute, University of Antwerp, Antwerp, Belgium

47 Rheumatology and Immunology Unit, Internal Medicine Department, Mansoura University, Mansoura, Egypt

48 Department of Internal Medicine, Faculty of Medicine, Horus University, New Damietta, Egypt

49 Department of Rheumatology, Faculty of Medicine Ankara City Hospital, Ankara Yıldırım Beyazıt University, Ankara, Turkey

50 Joint Academic Rheumatology Program, First Department of Propaedeutic and Internal Medicine, National and Kapodistrian University of Athens, Mikras Asias Street 75 Goudi, 11527 Athens, Greece

51 Rheumatology and Clinical Immunology, Spedali Civili and Department of Clinical and Experimental Sciences, University of Brescia, [European Reference Network (ERN) for Rare Immunodeficiency, Autoinflammatory and Autoimmune Diseases (RITA) Center], Brescia, Italy

52 U.O. Medicina Generale, Ospedale San Paolo di Civitavecchia, ASL Roma 4, Civitavecchia, Rome, Italy

53 Unit of Pediatric Rheumatology, ASST Gaetano Pini-CTO, Milan, Italy

54 UOC di Reumatologia, Azienda Ospedaliera San Camillo Forlanini, Rome, Italy

55 Department of Biomedical and Clinical Sciences Luigi Sacco, Luigi Sacco Hospital, University of Milan, Milan, Italy

56 Internal Medicine, Fatebenefratelli Hospital, Milan, Italy

57 Department of Biomedical and Clinical Sciences, University of Milan, Milan, Italy

58 Department of Life, Health & Environmental Sciences and Internal Medicine and Nephrology Unit, Department of Medicine, University of L'Aquila and ASL Avezzano-Sulmona-L'Aquila, San Salvatore Hospital, L'Aquila, Italy

59 Ophthalmology Unit, Department of Medicine, Surgery and Neurosciences, University of Siena and Azienda Ospedaliero-Universitaria Senese [European Reference Network (ERN) for Rare Immunodeficiency, Autoinflammatory and Autoimmune Diseases (RITA) Center], Siena, Italy

60 Bioengineering and Biomedical Data Science Lab, Department of Medical Biotechnologies, University of Siena, Siena, Italy

61 Policlinico "Le Scotte", Viale Bracci 1, 53100 Siena, Italy