Differentiating Macrophage Activation Syndrome in Systemic Juvenile Idiopathic Arthritis from Other Forms of Hemophagocytic Lymphohistiocytosis

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Objectives To identify measures distinguishing macrophage activation syndrome (MAS) in systemic juvenile idiopathic arthritis (sJIA) from familial hemophagocytic lymphohistiocytosis (FHL) and virus-associated hemophagocytic lymphohistiocytosis (VA-HLH) and to define appropriate cutoff values. To evaluate suggested dynamic measures differentiating MAS in patients with sJIA from sJIA flares.

Study design In a cohort of patients referred for evaluation of hemophagocytic lymphohistiocytosis, we identified 27 patients with sJIA and MAS (MAS/sJIA) fulfilling the criteria of the proposed preliminary diagnostic guideline for the diagnosis of MAS in sJIA. Ten measures at diagnosis were compared between the MAS/sJIA group and 90 patients with FHL and 42 patients with VA-HLH, and cutoff values were determined. In addition, 5 measures were analyzed for significant change from before MAS until MAS diagnosis.

Results Neutrophil count and C-reactive protein were significantly higher in patients with MAS/sJIA compared with patients with FHL and patients with VA-HLH, with 1.8 $\times$ 10^9/L neutrophils (sensitivity 85%, specificity 83%) and 90 mg/L C-reactive protein (74%, 89%) as cutoff values. Soluble CD25 <7900 U/L (79%, 76%) indicated MAS/sJIA rather than FHL/VA-HLH. Platelet (−59%) and white blood cell count (−46%) displayed a significant decrease, and neutrophil count (−35%) and fibrinogen (−28%) showed a trend during the development of MAS. However, a substantial portion of patients had values at diagnosis of MAS within or above the normal range for white blood cells (84%), neutrophils (77%), platelets (26%), and fibrinogen (71%).

Conclusion Readily available measures can rapidly differentiate between MAS/sJIA and FHL/VA-HLH. The findings substantiate that a decline of measures may facilitate the distinction of MAS from flares of sJIA. *(J Pediatr 2013;162:1245-51)*.

Acquired hemophagocytic lymphohistiocytosis (HLH) is a severe complication of autoimmune and autoinflammatory disease. By convention, this type of HLH is termed macrophage activation syndrome (MAS).[1] It is commonly associated with systemic juvenile idiopathic arthritis (sJIA) but also has been described in systemic lupus erythematosus,[2] inflammatory bowel disease,[3] and Kawasaki disease.[4] The condition is potentially fatal[5] and requires rapid recognition to initiate prompt treatment and prevent deleterious outcome. However, particularly in sJIA, the diagnosis is frequently difficult to make, and there is no gold standard to identify the condition with high sensitivity and specificity.

MAS can be the initial disease presentation of sJIA. In these cases, clinical signs such as arthritis frequently appear only later. This renders the distinction between MAS and hereditary HLH or acquired virus-associated HLH (VA-HLH) particularly difficult. Due to recent advances in the description of HLH-related gene defects, most patients with hereditary HLH can be identified through genetic analysis.[6] Flow cytometric analyses allow detection of a hereditary form in most cases within 48-72 hours. Intracellular perforin, SLAM-associated protein, and x-linked inhibitor of apoptosis can be stained, and reduced or absent appearance of CD107 on the surface of natural killer (NK) cells after stimulation indicates genetic defects of lytic granule transport and release (CD107 degranulation assay).[7] However, these investigations are not always easily available. Furthermore, the presence of a viral infection such as Epstein-Barr virus (EBV) and cytomegalovirus in a patient with HLH does not allow classification of the disease as VA-HLH. Viral infections not only can trigger HLH in otherwise healthy individuals but also can set

AUC	Area under the curve	PDG	Preliminary diagnostic guideline
CRP	C-reactive protein	ROC	Receiver operating characteristic
EBV	Epstein-Barr virus	sCD	Soluble CD
FHL	Familial hemophagocytic lymphohistiocytosis	sJIA	Systemic juvenile idiopathic arthritis
HLH	Hemophagocytic lymphohistiocytosis	VA-HLH	Virus-associated hemophagocytic lymphohistiocytosis
MAS	Macrophage activation syndrome	WBC	White blood cell
NK	Natural killer		

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The authors declare no conflicts of interest.

0022-3476/$ - see front matter. http://dx.doi.org/10.1016/j.jpeds.2012.11.081

off or exacerbate an episode in patients with genetic disease predisposing to HLH or with MAS in autoimmune and autoinflammatory conditions.[8,9] In this study, we established routine laboratory measures that help to distinguish sJIA-associated MAS from familial HLH (FHL) and VA-HLH.

Another clinical challenge is the proper distinction between a flare of sJIA and the development of sJIA-associated MAS, because the 2 conditions have overlapping features. For HLH, the HLH-2004 criteria have been established by the Histiocyte Society.[10] However, patients with autoinflammatory disease including sJIA usually display high white blood cell (WBC), neutrophil, and platelet counts and fibrinogen levels, as a feature of disease activity. A drop of these measures from elevated to normal values in an ongoing disease flare may indicate MAS,[9] which will not be interpreted correctly when using the HLH-2004 criteria. To address this problem, a preliminary diagnostic guideline (PDG) for MAS was suggested, based on the analysis of a cohort of patients with MAS compared with a group of patients with an acute flare of sJIA.[11] According to the PDG, the diagnosis of MAS requires the presence of ≥2 laboratory criteria or of ≥2 clinical and/or laboratory criteria: platelet count $<262 \times 10^9$/L, aspartate aminotransferase >59 U/L, WBC $<4 \times 10^9$/L, fibrinogen <2.5 g/L, hepatomegaly, neurologic symptoms, and hemorrhages. In this study, the fulfillment of the PDG criteria was mandatory for inclusion in the cohort of patients with MAS. We corroborated the preliminary findings of others[9,12,13] that the dynamics of measures during the development of MAS can help to differentiate MAS from flares of sJIA.

Methods

Patient data were retrieved from the database of the German national HLH study center, to which patients are referred from Germany, Austria, and Switzerland. Patients with rheumatic disease are not routinely reported, but patients are registered if the center is contacted due to uncertainty about the diagnosis and/or treatment of MAS or if MAS is the presenting feature of a condition, such as sJIA. Diagnosis of MAS or HLH was made between 1992 and 2010. Data were obtained at diagnosis before the start of immunosuppressive therapy for MAS or HLH. In cases of known sJIA with continuous immunomodulatory therapy, data were recorded before MAS-directed therapy was commenced.

Forty-seven patients <18 years of age with suspected MAS in sJIA were identified (MAS/sJIA group). We excluded 17 patients who on revision did not fulfill the revised International League of Associations for Rheumatism criteria for sJIA,[12] 1 patient who did not fulfill the PDG criteria,[11] and 2 patients for whom data were insufficient. Overall, 27 patients were included in the analysis. Studies HLH-94 and HLH-2004 were approved by the Ethics Committee of the Hamburg Chamber of Physicians; informed consent was obtained from the legal guardians.

In 90 patients with a genetic diagnosis of FHL, data obtained at diagnosis of HLH fulfilling the HLH-2004 criteria[10] were analyzed as controls (FHL group). In addition, 42 patients with VA-HLH (VA-HLH group) were studied, defined by fulfillment of the HLH-2004 criteria,[10] detection of EBV, cytomegalovirus, parvovirus B19, human herpes virus 6, or adenovirus by polymerase chain reaction or unequivocal serological analysis, and absence of relapse for >1 year. Patients with a genetic disorder predisposing to HLH, parental consanguinity, familial disease, abnormal perforin expression or abnormal degranulation of NK cells, signs of autoimmune or autoinflammatory disease, or *Leishmania* infection were excluded in the VA-HLH group.

Statistical Analyses

In a first step, the natural logarithms of the mean of hemoglobin level; WBC, neutrophil, and platelet counts; serum ferritin; fibrinogen; triglycerides; soluble CD (sCD)25 (Immulite immunoassay system, Siemens Healthcare Diagnostics, New York, New York); and C-reactive protein (CRP) at diagnosis of MAS or HLH were compared between (1) MAS/sJIA and FHL and (2) MAS/sJIA and VA-HLH using a 1-way ANCOVA with adjustment for age at onset and post-hoc testing. In addition, this step was performed for age at onset using ANOVA. Laboratory measures of FHL and VA-HLH were in a similar range. Thus, in a second step, FHL and VA-HLH laboratory data were pooled (FHL/VA-HLH) and a comparison was performed (3) between MAS/sJIA and FHL/VA-HLH using a 1-way ANCOVA with adjustment for age at onset. Differences were considered significant at $P < .0017$ after Bonferroni adjustment for 30 comparisons corresponding to an overall level of significance of .05. We tested for sex as a confounding variable by including this factor as an adjusting covariable in a first step. Because sex had no significant impact on the results, this factor was eliminated for further conclusions (backward selection). Finally, receiver operating characteristic (ROC) curves were calculated to determine cutoff values for the differentiation between MAS/sJIA and FHL/VA-HLH. Values for which the sum of sensitivity and specificity reached the maximum were taken as cutoffs. Measures that did not allow identification of 1 single cutoff value with high separation accuracy were excluded.

In addition, we calculated the change in WBC, neutrophil, and platelet counts, fibrinogen, and CRP. To this end, data for our patients were analyzed at 2 time points: before the development of MAS and at diagnosis before the start of MAS-directed therapy. From each patient, the only data included were those for which both values were available. The overall difference was described by the median of the proportionate change of each patient. Significance of differences was assumed at $P < .01$, as determined with a 2-sided Student t test for matched pairs after Bonferroni adjustment for 5 comparisons, corresponding to an overall level of significance of .05. To compare the relevance of the dynamic measures to absolute figures, the number of patients with WBC, neutrophil, and platelet counts and fibrinogen at diagnosis within or above the normal range was determined (including only those data points that had been used previously in the evaluation of the dynamic measures).

Finally, the sensitivity of each single criterion of the HLH-2004 criteria[10] and the proportion of patients fulfilling each single criterion of the PDG[11] were determined.

Statistical analyses were executed using PASW Statistics 18 (SPSS, Chicago, Illinois).

Results

Characteristics of Patients with MAS and sJIA

The median age at the time of diagnosis of MAS in patients with MAS/sJIA was 10.0 years, and 59% were girls. In 22 of the 27 patients of the cohort, MAS was the first manifestation of sJIA. Arthritis was present initially in 17 and appeared only later in the course of disease in 5 of 22. In 5 patients, MAS manifested in previously diagnosed sJIA. The mean time between manifestation of sJIA and MAS in this subgroup was 5 years (range 0.75-10 years). Acute EBV infection was identified in 3 patients. No other viruses were detected. Three patients (11%) died as a consequence of MAS. Evaluation of treatment was not an objective of this study.

Comparison of MAS/sJIA versus FHL and VA-HLH and Identification of Cutoff Values

Laboratory data and age at diagnosis of patients with MAS/sJIA were compared with those of patients with: (1) FHL; (2) VA-HLH; and (3) the pooled cohort of FHL/VA-HLH (Table I). The FHL cohort consisted of 26 patients with FHL2, 31 with FHL3, 6 with FHL4, and 27 with FHL5 (median age 0.32 year). The median age in the VA-HLH group was 4.7 years. A significantly higher level of neutrophil count and CRP was found in patients with MAS/sJIA in all 3 comparisons. Ferritin was significantly higher in comparison with FHL and with the pooled cohort. As expected, the age at onset in FHL was significantly lower than in MAS/sJIA. ROC curves were calculated to determine a cutoff value for the differentiation between MAS/sJIA and FHL/VA-HLH (Table II). An area under the curve (AUC) >0.75, indicating high separation accuracy, was found for neutrophil count, CRP, sCD25, and hemoglobin. Despite significance in ANCOVA, the AUC of ferritin was low. Neutrophil count >1.8 $\times$ 10^9/L (sensitivity 85%, specificity 83%), CRP >90 mg/L (74%, 89%), and sCD25 <7900 U/mL (79%, 76%) indicate MAS/sJIA rather than FHL/VA-HLH.

Evaluation of Potential Dynamic Measures

We then evaluated the dynamics of WBC, neutrophil, and platelet counts, fibrinogen, and CRP until the diagnosis of MAS (Table III). The mean number of days between the first (before the diagnosis of MAS) and the second (at the diagnosis of MAS) measurements was 6.3 days (±SD, 2.9 days). In the median, WBC and platelet counts significantly declined between the 2 time points; neutrophil count and fibrinogen showed a downward trend; and the change in CRP was inconsistent. It is noteworthy, first, that before the diagnosis of MAS, the median values for WBC (15.9 $\times$ 10^9/L, n = 19, normal range 5-15 $\times$ 10^9/L), neutrophil count (9.7 $\times$ 10^9/L, n = 13, normal range 1.5-8.5 $\times$ 10^9/L), and fibrinogen (4.3 g/L, n = 7, normal range 1.5-3.5 g/L) were above the upper limit of normal and the values at diagnosis were in the normal range (7.8 $\times$ 10^9/L, 4.2 $\times$ 10^9/L, and 3.4 g/L, respectively). Second, only 4 of 19 patients of this cohort met the PDG threshold for WBC (<4 $\times$ 10^9/L) and only 2 of 7 patients had a fibrinogen level below the PDG cutoff (<2.5 g/L). Platelet count in this cohort dropped from a median level in the normal range (216 $\times$ 10^9/L, n = 19, normal range 150-400 $\times$ 10^9/L) to a value below the norm (96 $\times$ 10^9/L). However, 26% of patients in this cohort still had a platelet count above the lower limit of normal at diagnosis of MAS. In consequence, for the diagnosis of MAS, the change from an elevated to a normal level appeared to be more reliable than the absolute values for WBC, neutrophil, and platelet counts and fibrinogen.

Evaluation of Current Diagnostic Criteria in the MAS/sJIA Cohort

The sensitivity (Table IV) of the overall HLH-2004 criteria (ie, fulfillment of ≥5 of 8 criteria) was only 67%, with high sensitivity for the markers of inflammation ferritin (96%) and sCD25 (89%). The sensitivity rendered by the HLH-2004 cutoff values was particularly low for neutrophil count (4%), cytopenia of ≥2 cell lines (37%), and fibrinogen (46%). Specificity was not determined in the absence of a negative control group.

Even though by definition all patients of the MAS/sJIA group met the overall PDG, the proportion of patients fulfilling each single criterion ranged widely. All 27 patients met the threshold for platelet count; only 4 had hemorrhages.

Discussion

MAS and FHL/VA-HLH share several features that not only comprise the substantial clinical overlap but include genetic and functional abnormalities, such as low perforin expression, perforin, and Munc13-4 sequence variants and reduced NK cytotoxicity.[7,14-17] However, current treatment recommendations for MAS and FHL/VA-HLH differ substantially.[1,5,10,18-22] Thus, as the main focus of this study, measures were analyzed for their potential to discriminate between MAS in sJIA, on the one hand, and FHL/VA-HLH, on the other, to allow rapid decision making regarding therapy. Second, the dynamics of measures until the diagnosis of MAS were analyzed to test their ability to identify the development of MAS in patients with sJIA. In addition, the validity of the HLH-2004 criteria[10] was evaluated in patients in this MAS cohort.

Because the German HLH study center is supported by the German Society for Oncology and Hematology, more patients were registered by hematologists than by rheumatologists. Consequently, this cohort has a high proportion of patients with MAS in not-yet-diagnosed sJIA (77%). Because these episodes are particularly difficult to differentiate from other forms of HLH, the cohort is especially suitable to test for measures differentiating between MAS/sJIA, on the one hand, and FHL/VA-HLH, on the other. However, a selection

Table I. Comparison of measures in MAS/sJIA, FHL, and VA-HLH

Measures	Total			MAS			FHL			VA-HLH			FHL + VA-HLH	
Hemoglobin, g/dL														
25th, 50th, 75th percentile	6.4	7.7	9.3	8.1	9.4	11.1	6.0	7.1	8.5	7.0	8.3	9.1	6.3	7.4	8.7
n		159			27			90			42			132	
P value vs MAS								.012			.005			.003	
WBC count, $\times 10^9$/L															
25th, 50th, 75th percentile	2.3	3.9	7.1	3.2	7.1	12.0	2.3	3.7	5.8	1.9	3.9	8.2	2.3	3.8	6.0
n		128			27			67			34			101	
P value vs MAS								.004			.006			.002	
Neutrophil count, $\times 10^9$/L															
25th, 50th, 75th percentile	0.4	0.8	2.0	2.0	4.0	8.5	0.2	0.6	1.2	0.6	1.5	2.4	0.3	0.7	1.5
n		141			20			83			37			121	
P value vs MAS								<.001*			.001*			<.001*	
Platelet count, $\times 10^9$/L															
25th, 50th, 75th percentile, $\times 10^9$/L	20	44	81	45	81	96	16	28	51	41	73	101	18	40	74
n		159			27			90			42			132	
P value vs MAS								.023			.961			.362	
Ferritin, μg/L															
25th, 50th, 75th percentile	1590	4950	12 490	4820	8720	30 160	1420	3070	10 750	1590	5310	10 750	1470	3760	10 700
n		146			26			82			38			120	
P value vs MAS								<.001*			.01			.001*	
Fibrinogen, g/L															
25th, 50th, 75th percentile	0.7	1.15	2.1	1.2	2.3	3.6	0.5	0.9	1.6	1.0	1.4	2.3	0.7	1.0	1.8
n		147			24			84			39			123	
P value vs MAS								.048			.465			.196	
Triglycerides, mmol/L															
25th, 50th, 75th percentile	2.6	3.8	5.3	2.6	3.3	4.7	2.7	3.9	5.4	2.4	3.7	5.7	2.6	3.8	5.4
n		138			20			80			38			118	
P value vs MAS								.699			.71			.913	
sCD25, U/mL															
25th, 50th, 75th percentile	7100	14 100	23 700	3000	5000	7800	11 500	19 300	30 400	6600	9900	15 300	8100	15 000	25 200
n		112			19			59			34			93	
P value vs MAS								.009			.034			.014	
CRP, mg/L															
25th, 50th, 75th percentile	15	44	89	75	140	252	12	28	57	14	39	79	13	30	63
n		120			27			55			38			93	
P value vs MAS								<.001*			<.001*			<.001*	
Age at onset, y															
25th, 50th, 75th percentile	0.3	1.4	7.0	5	10	15.4	0.2	0.3	1.3	1.8	4.7	9.6		NA	
n		156			27			87			42				
P value vs MAS								<.001*			.031				

NA, not applicable.

Data were compared between patients with sJIA and MAS with: (1) FHL; (2) VA-HLH; and (3) a pooled cohort of FHL and VA-HLH (except for age at onset). Significant difference (*) was assumed at a *P* value <.0017, with Bonferroni correction for multiple testing, corresponding to an overall level of significance of .05.

Table II. Determination of cutoff values

	Neutrophil count	CRP	sCD25	Hemoglobin	Platelet count	Fibrinogen	Ferritin	WBC count	Triglycerides
ROC–AUC	0.89	0.87	0.79	0.76	0.71	0.71	0.69	0.68	0.58
Maximum sensitivity + specificity	1.68	1.63	1.55	1.44	1.38	1.38	1.36	1.35	1.22
Cutoff value (indicating MAS)	$\geq 1.8 \times 10^9$/L	≥90 mg/L	≤7900 U/mL	—	—	—	—	—	—
Sensitivity (MAS)	0.85	0.74	0.79	—	—	—	—	—	—
Specificity (MAS)	0.83	0.89	0.76	—	—	—	—	—	—

An ROC analysis was performed to determine cutoff values that differentiate between MAS and the pooled cohort of FHL and VA-HLH. A ROC-AUC of 1.0 indicates perfect separation accuracy and 0.5 indicates absence of discriminatory capacity. Values rendering the highest sensitivity plus specificity were chosen as cutoffs for neutrophil count, CRP, and sCD25.

bias toward patients at the more severe end of the disease spectrum, with substantial hematologic derangement, can be assumed. If detailed in previous publications,[5,13,18,23] 0-67% of MAS episodes occurred at the intial presentation of sJIA Mortality in the MAS/sJIA cohort (11%) is comparable with previous reports of 0%-29%.[5,9,13,18,23,24] The evaluation of treatment was not the aim of this study.

Routine laboratory data could be identified that facilitate the differentiation of MAS in patients with sJIA from FHL or acquired VA-HLH. Because MAS may present at the first manifestation of sJIA and because arthritis may initially be absent, this distinction can be difficult to make based on signs and symptoms only. The identified criteria may serve as a tool to distinguish between MAS/sJIA and VA-HLH. This is especially challenging due to the substantial overlap in the distribution of age at onset (Table I) and the lack of pathognomonic features (in contrast to FHL with reduced perforin expression or NK degranulation and unequivocal mutations). The measures with the highest AUC in the ROC analysis were neutrophil count and CRP, indicating best separation accuracy. These measures are typically high in sJIA and, in the event of MAS, are initially still elevated, whereas they are usually low in FHL and VA-HLH. Consequently, values above the identified cutoff of 1.8×10^9/L neutrophils and 90 mg/L CRP indicate MAS/sJIA rather than FHL or VA-HLH. It should be noted that in patients with FHL/VA-HLH, systemic infection may occur due to neutropenia resulting in elevated CRP.

Even though the comparison of sCD25 values just missed the level of significance in the age-adjusted ANCOVA after Bonferroni correction, the ROC analysis rendered cutoff value with good separation accuracy (7900 U/mL), indicating a higher degree of T-cell activation in FHL/VA-HLH. Physiologic sCD25 plasma levels decline during childhood,[25] with a mean of 540 U/mL at the age of 2 years and 320 U/mL at the age of 16 years when using the Immulite detection system (Siemens, Erlangen, Germany).[26] However, because the levels in our cohorts were considerably higher, the confounding effect is likely to be minimal. Despite significant differences in ferritin level between MAS/sJIA and FHL/VA-HLH, the AUC of the ROC was low, implying low separation accuracy of this analyte. Thus, no cutoff value could be determined. As expected, age at onset does not differentiate between MAS/sJIA and VA-HLH. In a previous report, the levels of neopterin and interleukin-18 were reported to differentiate between sJIA/MAS and EBV-associated HLH.[27] However, these measures are not universally available.

The criteria identified in this study may give guidance as to which patients should be tested for defects of degranulation or expression of HLH-associated proteins and subsequently genetic defects to identify hereditary HLH.[7] However, to fully exclude hereditary disease, functional and genetic testing should as well be considered in patients with suspected MAS/sJIA according to the cited data, particularly if arthritis is initially absent.

It has been suggested that the dynamics of measures may more precisely distinguish MAS/sJIA from flares of patients with sJIA than will absolute values. Our data demonstrate that decreasing platelet (median reduction of −59%) and WBC (−46%) counts are significant indicators of MAS. Decreasing neutrophil count and fibrinogen levels may prove to be significant in larger patient numbers. Notably, median WBC and neutrophil counts and fibrinogen were above the upper limit of normal before the MAS episode and within (and not below) the normal range at the diagnosis of MAS. This is supported by the finding that few patients met the

Table III. Dynamics of measures during the development of MAS

	WBC count	Neutrophil count	Platelet count	Fibrinogen	CRP
N	19	13	19	7	16
Before MAS (median)	15.9×10^9/L	9.7×10^9/L	216×10^9/L	4.3 g/L	130 mg/L
At MAS (median)	7.8×10^9/L	4.2×10^9/L	96×10^9/L	3.4 g/L	122 mg/L
Difference (median)	−46%	−35%	−59%	−28%	−8%
P value	<.001*	.022	.002*	.117	.309
Normal range	$5\text{-}15 \times 10^9$/L	$1.5\text{-}8.5 \times 10^9$/L	$150\text{-}400 \times 10^9$/L	1.5-3.5 g/L	NA
Patients >LLN, %	84%	77%	26%	71%	NA

LLN, lower limit of normal.
The dynamics of routine markers of sJIA disease activity were analyzed in the MAS/sJIA group. The mean number of days between the first (before the diagnosis of MAS) and the second (at the diagnosis of MAS) measurement was 6.3 d (±2.9 d SD). WBC and platelet counts displayed a significant decline. Of note, a substantial proportion of patients had values within the norm at diagnosis, indicating an important role for assessing change.

Table IV. Evaluation of current diagnostic criteria

Criterion (cutoff)	n	Fulfilled, %	25th Percentile	Median	75th Percentile
HLH-2004 criteria					
Overall (≥5 criteria)	27	67	—	—	—
Fever (yes)	27	100	—	—	—
Splenomegaly (yes)	27	67	—	—	—
Hemoglobin (<9 g/dL)	27	37	8.1	9.4	11.1
Neutrophil count (<1 × 10^9/L)	27	4	2.0	4.0	8.5
Platelet count (<100 × 10^9/L)	27	78	45	81	96
Cytopenia (≥2 lines, previous rows)	27	37	—	—	—
Ferritin (>500 μg/L)	26	96	4820	8720	30 160
Fibrinogen (<1.5 g/L)	24	46	1.2	2.3	3.6
Triglycerides (>3.0 mmol/L)	20	60	2.6	3.3	4.7
sCD25 (>2400 U/mL)	19	89	2970	4980	7830
Hemophagocytosis (yes)	23	74	—	—	—
PDG					
Overall, mandatory for inclusion	27	100	—	—	—
Platelet count (<262 × 10^9/L)	27	100	45	81	96
ASAT (>59 U/L)	27	93	141	213	499
WBC (<4 × 10^9/L)	27	30	3.2	7.1	12
Fibrinogen (<2,5 g/L)	24	58	1.2	2.3	3.6
Hepatomegaly (yes)	27	67	—	—	—
Neurologic symptoms (yes)	27	33	—	—	—
Hemorrhages (yes)	27	15	—	—	—

ASAT, aspartate aminotransferase.
The proportion of patients fulfilling the PDG[11] and the HLH-2004 criteria[10] was evaluated in an MAS cohort.

absolute PDG criterion for WBC and fibrinogen. Even though platelet count in the median started in the norm and dropped to a level below the lower limit of normal, a relevant number of patients indeed had a platelet count within the normal range at diagnosis (Table III). All patients invariably met the relatively high cutoff for platelet count of the PDG (<262 × 10^9/L). Due to the absence of a control group of patients with sJIA without MAS or other conditions, the specificity could not be determined and thus no cutoff values for a relative reduction can be suggested. However, these findings support that a decline of the analyzed measures should raise strong suspicion of incipient MAS. A reduction in CRP, in contrast, does not seem to be a consistent feature of MAS. In a Delphi survey, 232 pediatric rheumatologists subjectively ranked falling platelet and WBC counts in the top 5 most important features to identify MAS in sJIA.[12] In another study with 37 patients, a drop in platelet, WBC, and neutrophil counts and fibrinogen was significant,[13] and in a small series of 8 patients, the most striking reduction was noted for platelet count.[9]

Several other investigations have been suggested to differentiate MAS from flares of sJIA: elevated sCD163, reduced C3 and C4, and more sophisticated analyses, such as gene expression profiling, were proposed as potential disease markers.[28-30] It has been assumed that the presence of hemophagocytosis alone represents a subclinical form of MAS,[31] implying that the mere finding of hemophagocytosis is sufficient for the diagnosis of MAS. In our study, 74% of patients with MAS displayed hemophagocytosis. In patients treated according to the HLH-1994 study, prevalence was 92%.[20] From personal experience, the finding of low-level hemophagocytosis represents a rather unspecific finding and may be present in several conditions. Eventually, only a set of dynamic and absolute values and findings can well characterize and define MAS.

The results of this study underscore that HLH-2004 criteria should not be used to diagnose MAS, because their sensitivity is low. As outlined in the original publication of the PDG,[11] the proportion of patients fulfilling each single criterion of the PDG is highly variable. Of note, some clinical features that are ranked in the top half of 28 measures of the Delphi survey[12] and that are included in the PDG, such as central nervous system dysfunction and hemorrhages, are manifestations at a late stage of MAS. Our data corroborate that in incipient MAS, their sensitivity is low; however, if present, they are highly specific.[11] The primary data of our MAS/sJIA cohort will be included in a large ongoing global evaluation of the diagnosis of the international pediatric rheumatology networks to achieve more statistical power with larger patient numbers for the objective of differentiating MAS from flares of sJIA. ■

The authors would like to thank Professor Stephan Ehl, MD, for functional analyses to exclude hereditary disease and critical revision of the manuscript, Udo zur Stadt, PhD, for genetic testing to exclude hereditary disease, and Elisabeth Weißbarth-Riedel, MD (receives financial support from Novartis), for critical revision of the manuscript.

Submitted for publication Jul 15, 2012; last revision received Sep 10, 2012; accepted Nov 29, 2012.

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