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Performance of Current Guidelines for Diagnosis of Macrophage Activation Syndrome Complicating Systemic Juvenile Idiopathic Arthritis

Sergio Davì,[1] Francesca Minoia,[1] Angela Pistorio,[2] AnnaCarin Horne,[3] Alessandro Consolaro,[2] Silvia Rosina,[1] Francesca Bovis,[1] Rolando Cimaz,[4] Maria Luz Gamir,[5] Norman T. Ilowite,[6] Isabelle Kone-Paut,[7] Sheila Knupp Feitosa de Oliveira,[8] Deborah McCurdy,[9] Clovis Artur Silva,[10] Flavio Sztajnbok,[11] Elena Tsitsami,[12] Erbil Unsal,[13] Jennifer E. Weiss,[14] Nico Wulffraat,[15] Mario Abinun,[16] Amita Aggarwal,[17] Maria Teresa Apaz,[18] Itziar Astigarraga,[19] Fabrizia Corona,[20] Ruben Cuttica,[21] Gianfranco D'Angelo,[22] Eli M. Eisenstein,[23] Soad Hashad,[24] Loredana Lepore,[25] Velma Mulaosmanovic,[26] Susan Nielsen,[27] Sampath Prahalad,[28] Donato Rigante,[29] Valda Stanevicha,[30] Gary Sterba,[31] Gordana Susic,[32] Syuji Takei,[33] Ralf Trauzeddel,[34] Mabruka Zletni,[24] Nicolino Ruperto,[2] Alberto Martini,[35] Randy Q. Cron,[36] and Angelo Ravelli,[35] on behalf of the Paediatric Rheumatology International Trials Organisation, the Childhood Arthritis and Rheumatology Research Alliance, the Pediatric Rheumatology Collaborative Study Group, and the Histiocyte Society

Objective. **To compare the capacity of the 2004 diagnostic guidelines for hemophagocytic lymphohistiocytosis (HLH-2004) with the capacity of the preliminary diagnostic guidelines for systemic juvenile idiopathic arthritis (JIA)–associated macrophage activation syndrome (MAS) to discriminate MAS complicating systemic JIA from 2 potentially confusable conditions, represented by active systemic JIA without MAS and systemic infection.**

[1]Sergio Davì, MD, Francesca Minoia, MD, Silvia Rosina, MD, Francesca Bovis, BiolD: Università degli Studi di Genova, Genoa, Italy; [2]Angela Pistorio, MD, PhD, Alessandro Consolaro, MD, PhD, Nicolino Ruperto, MD, MPH: IRCCS G. Gaslini, Genoa, Italy; [3]AnnaCarin Horne, MD, PhD: Karolinska University Hospital Solna, Stockholm, Sweden; [4]Rolando Cimaz, MD: Ospedale Pediatrico A. Meyer, Florence, Italy; [5]Maria Luz Gamir, MD: Hospital Ramon y Cajal, Madrid, Spain; [6]Norman T. Ilowite, MD: Albert Einstein College of Medicine and Children's Hospital at Montefiore, Bronx, New York; [7]Isabelle Kone-Paut, MD, PhD: AP-HP, Centre Hospitalier Universitaire Le Kremlin Bicêtre, Le Kremlin Bicêtre, France; [8]Sheila Knupp Feitosa de Oliveira, MD: Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; [9]Deborah McCurdy, MD: University of California, Los Angeles and Mattel Children's Health Center UCLA, Los Angeles, California; [10]Clovis Artur Silva, MD: Universidade de São Paulo, São Paulo, Brazil; [11]Flavio Sztajnbok, MD: Hospital Universitario Pedro Ernesto, Rio de Janeiro, Brazil; [12]Elena Tsitsami, MD: University of Athens Medical School and Children's Hospital Aghia Sofia, Athens, Greece; [13]Erbil Unsal, MD: Dokuz Eylül University Medical School, Izmir, Turkey; [14]Jennifer E. Weiss, MD: New Jersey Medical School and Hackensack University Medical Center, Hackensack, New Jersey; [15]Nico Wulffraat, MD: Wilhelmina Children's Hospital and University Medical Center Utrecht, Utrecht, The Netherlands; [16]Mario Abinun, MD: Great North Children's Hospital, Newcastle, UK; [17]Amita Aggarwal, MD: Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India; [18]Maria Teresa Apaz, MD: Universidad Católica de Córdoba, Córdoba, Argentina; [19]Itziar Astigarraga, MD, PhD: Hospital de Cruces, Bilbao, Spain; [20]Fabrizia Corona, MD: Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy; [21]Ruben Cuttica, MD: Hospital General de Ninos Pedro de Elizalde, Buenos Aires, Argentina; [22]Gianfranco D'Angelo, MD: Ospedale Pediatrico G. Salesi Ancona, Ancona, Italy; [23]Eli M. Eisenstein, MD: Hadassah–Hebrew University Medical Center, Jerusalem, Israel; [24]Soad Hashad, MD, Mabruka Zletni, MD: Tripoli Children's Hospital, Tripoli, Libya; [25]Loredana Lepore, MD: IRCCS Materno Infantile Burlo Garofolo, Trieste, Italy; [26]Velma Mulaosmanovic, MD: Children's Hospital University Clinical Center Sarajevo, Sarajevo, Bosnia and Herzegovina; [27]Susan Nielsen, MD: Juliane Marie Centret and Rigshospitalet, Copenhagen, Denmark; [28]Sampath Prahalad, MD: Emory University and Children's Healthcare of Atlanta, Atlanta, Georgia; [29]Donato Rigante, MD: Università Cattolica del Sacro Cuore, Roma, Italy; [30]Valda Stanevicha, MD: Riga Stradins University, Riga, Latvia; [31]Gary Sterba, MD: Mount Sinai Medical Center, Miami Beach, Florida; [32]Gordana Susic, MD: Institute of Rheumatology, Belgrade, Serbia; [33]Syuji Takei, MD: Kagoshima University Hospital, Kagoshima, Japan; [34]Ralf Trauzeddel, MD: Helios Kliniken Berlin, Berlin, Germany; [34]Alberto Martini, MD, Angelo Ravelli, MD: IRCCS G. Gaslini and Università degli Studi di Genova, Genoa, Italy; [35]Randy Q. Cron, MD, PhD: University of Alabama at Birmingham.

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Address correspondence to Angelo Ravelli, MD, Pediatria II, IRCCS G. Gaslini, Largo G. Gaslini 5, Genoa 16147, Italy. E-mail: angeloravelli@ospedale-gaslini.ge.it.

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Methods. **International pediatric rheumatologists and hemato-oncologists were asked to retrospectively collect clinical information from patients with systemic JIA–associated MAS and confusable conditions. The ability of the guidelines to differentiate MAS from the control diseases was evaluated by calculating the sensitivity and specificity of each set of guidelines and the kappa statistics for concordance with the physician's diagnosis. Owing to the fact that not all patients were assessed for hemophagocytosis on bone marrow aspirates and given the lack of data on natural killer cell activity and soluble CD25 levels, the HLH-2004 guidelines were adapted to enable the diagnosis of MAS when 3 of 5 of the remaining items (3/5-adapted) or 4 of 5 of the remaining items (4/5-adapted) were present.**

Results. **The study sample included 362 patients with systemic JIA and MAS, 404 patients with active systemic JIA without MAS, and 345 patients with systemic infection. The best capacity to differentiate MAS from systemic JIA without MAS was found when the preliminary MAS guidelines were applied. The 3/5-adapted HLH-2004 guidelines performed better than the 4/5-adapted guidelines in distinguishing MAS from active systemic JIA without MAS. The 3/5-adapted HLH-2004 guidelines and the preliminary MAS guidelines with the addition of ferritin levels ≥500 ng/ml discriminated best between MAS and systemic infections.**

Conclusion. **The preliminary MAS guidelines showed the strongest ability to identify MAS in systemic JIA. The addition of hyperferritinemia enhanced their capacity to differentiate MAS from systemic infections. The HLH-2004 guidelines are likely not appropriate for identification of MAS in children with systemic JIA.**

Macrophage activation syndrome (MAS) is a potentially fatal complication of systemic juvenile idiopathic arthritis (JIA), whose pathophysiologic hallmark is an exaggerated but ineffective immune response involving excessive macrophage and T cell activation that produces high levels of proinflammatory cytokines (1,2). The cardinal clinical symptoms and signs of MAS are prolonged high fever, hepatosplenomegaly, neurologic dysfunction, and hemorrhagic manifestations. Characteristic laboratory abnormalities include pancytopenia, elevated levels of serum liver enzymes, triglycerides, lactate dehydrogenase, and ferritin, and low levels of fibrinogen. Although macrophage hemophagocytosis is often seen on bone marrow examination, this finding may be absent, particularly in the initial stages of the syndrome (3–6). The presence of hemophagocytosis may be governed by a relative paucity of the antiinflammatory cytokine interleukin-10 (IL-10) (7).

The estimated prevalence of MAS in systemic JIA is ~10%. However, recent evidence suggests that the syndrome may occur subclinically in an additional 30–40% of systemic JIA patients (8,9). Because MAS bears a close similarity to the group of histiocytic disorders belonging to hemophagocytic lymphohistiocytosis (HLH), it is currently classified among the secondary, or acquired, forms of HLH (10,11). Along these lines, patients with systemic JIA who develop MAS may share similar genetic propensities as those with primary HLH (2).

Timely diagnosis of MAS is critical to start therapy before the damage related to hypercytokinemia becomes irreversible. However, there is no single feature that is specific for MAS, including hemophagocytosis (12). Furthermore, MAS can be hard to distinguish from conditions that may present with overlapping features, such as flares of systemic JIA, sepsis or sepsis-like syndromes, or adverse effects of antiarthritis medications. Differentiation of MAS from these conditions is fundamental in selecting the appropriate therapeutic interventions in a timely manner.

The difficulties in making the diagnosis emphasize the importance of reliable criteria that could aid physicians in identifying MAS in its earliest stages and in distinguishing it from confusable conditions. Two sets of guidelines are currently available for diagnosing MAS in patients with systemic JIA. The recognition that the syndrome is clinically similar to HLH has led some to recommend the use of the 2004 diagnostic guidelines for HLH (HLH-2004), which were developed primarily for homozygous genetic disorders leading to hemophagocytosis (13). An alternative approach is based on the application of the preliminary diagnostic guidelines for MAS complicating systemic JIA, which were created through the analysis of a cohort of patients with MAS compared with a group of patients with a flare of systemic JIA (14). Although both guidelines are considered potentially suitable for detecting MAS in systemic JIA, it has been argued that each of them is affected by a number of potential shortcomings (2,11,15,16). However, the diagnostic performance of the 2 sets of guidelines has never been scrutinized using real patient data. For this reason, the aim of the present study was to compare the capacity of the HLH-2004 guidelines with the capacity of the preliminary MAS guidelines to differentiate systemic JIA–associated MAS from 2 potentially confusable conditions, represented by active systemic JIA without MAS and systemic infection.

PATIENTS AND METHODS

Study design and patient selection. Pediatric rheumatologists belonging to the Paediatric Rheumatology International Trials Organisation, the Pediatric Rheumatology Collaborative Study Group, and the Childhood Arthritis and Rheumatology Research Alliance, and pediatric hematooncologists belonging to the Histiocyte Society were contacted by e-mail and invited to participate in a retrospective cohort study of patients with systemic JIA–associated MAS and those with 2 conditions potentially confusable with MAS, represented by active systemic JIA without evidence of MAS and systemic infection, with data recorded in their hospital's database. To facilitate enrollment of patients with systemic infection, investigators were asked to involve infectious disease specialists practicing at their hospital.

To be included in the study, patients with MAS had to have been diagnosed as having systemic JIA according to the International League of Associations for Rheumatology (ILAR) (17) and to have had an episode of MAS diagnosed and treated as such by the attending physician. The diagnosis of MAS had to be based on the typical clinical and laboratory picture of the syndrome, irrespective of evidence of macrophage hemophagocytosis in the bone marrow aspirate. Patients with active systemic JIA without MAS should have also met the ILAR criteria for systemic JIA, but should not have any clinical or laboratory evidence of ongoing MAS. The systemic infection sample included patients who did not have systemic JIA and had an acute febrile infection requiring hospitalization. Because the clinical and laboratory picture of visceral leishmaniasis may be indistinguishable from that of MAS (18), patients with this infection were excluded.

Investigators were asked to include in the study only those patients seen after 2002. This time frame was chosen because the full awareness of the typical clinical and laboratory features of MAS was achieved in the early 2000s. Since some patients had multiple episodes of MAS, only the first episode had to be selected. Likewise, only one episode per patient had to be included for both patients with active systemic JIA without MAS and those with systemic infection.

The study protocol was approved by the Institutional Review Board at each participating center.

Data collection procedure. Investigators who agreed to participate in the study were asked to complete a structured case report form with each patient's anonymous data, and then to enter the data into a electronic web–based database developed and handled at the coordinating center (the IRCCS G. Gaslini of Genoa, Italy). For the purposes of the present study, the following information was collected from both patients with MAS and control patients: demographic data, clinical manifestations, laboratory parameters, and histopathologic features. Demographic data included sex and age at onset of the disease. Clinical and laboratory data included those parameters that are known to be most relevant for the diagnosis of MAS (19). In patients with MAS, clinical and laboratory data were collected at onset of the syndrome, that is, at the time when the first signs or symptoms consistent with MAS were detected. The histopathologic study investigators asked whether bone marrow aspirate or other biopsies were performed, and for those patients in whom any of these procedures were done, the investigators noted evidence of macrophage hemophagocytosis.

HLH-2004 diagnostic guidelines. According to the Histiocyte Society's updated diagnostic and therapeutic guidelines for HLH (13), a diagnosis of HLH can be established either by a molecular diagnosis with specific gene mutations associated with HLH or by meeting 5 of 8 clinical and laboratory diagnostic criteria for nonfamilial HLH. These criteria include the following features: fever, splenomegaly, peripheral blood cytopenias affecting at least 2 of 3 cell lineages, hypertriglyceridemia or hypofibrinogenemia, microscopic evidence of hemophagocytosis in the bone marrow, spleen, or lymph nodes, low or absent natural killer (NK) cell activity, elevated ferritin levels, and elevated soluble CD25 (sCD25; IL-2 receptor) levels. Because information about the presence of hemophagocytosis was not available for both control groups and because neither NK cell activity nor sCD25 levels were determined in all patients, the diagnostic rule was modified to enable the diagnosis of HLH when either 3 of 5 of the remaining criteria (3/5-adapted) or 4 of 5 of the remaining criteria (4/5-adapted) were met. Patients who did not meet the criteria for cytopenia and hypertriglyceridemia/hypofibrinogenemia, but lacked the minimum number of items necessary to assess the respective criterion, were excluded from the analysis.

Preliminary diagnostic guidelines for MAS complicating systemic JIA. According to the preliminary MAS guidelines (14), the diagnosis of MAS requires the presence of ≥ 2 laboratory criteria or ≥ 2 clinical and/or laboratory criteria. Laboratory criteria include a platelet count $\leq 262 \times 10^9$/liter, aspartate aminotransferase level >59 units/liter, white blood cell count $\leq 4.0 \times 10^9$/liter, and fibrinogen level ≤ 250 gm/liter. Clinical criteria include hepatomegaly, hemorrhagic manifestations, and central nervous system (CNS) dysfunction. The demonstration of macrophage hemophagocytosis on bone marrow aspirate is requested only in doubtful cases. The criteria were tested both in their original format and with the additional criterion of hyperferritinemia, which was defined at various thresholds (500, 1,000, and 2,000 ng/ml).

Statistical analysis. Quantitative data are presented as the median with interquartile range, and categorical data are presented as the absolute number with percentage. Comparison of quantitative variables between the MAS group and the control groups was done using the Mann-Whitney U test, whereas comparison of categorical variables was done using the chi-square test, or Fisher's exact test when the expected frequency was <5.

The ability of the HLH-2004 guidelines and the preliminary MAS guidelines to discriminate patients with MAS from control patients was evaluated by calculating the sensitivity of each set of guidelines (ability of the criteria to identify a patient as having MAS who had been diagnosed as having MAS by the attending physician) and specificity of each set of guidelines (ability of the criteria to identify a patient as not having MAS who had been diagnosed as having systemic JIA without MAS or systemic infection by the attending physician). The chance-corrected concordance between the diagnosis yielded by the criteria and the diagnosis made by the attending physician was assessed using Cohen's kappa statistics (20). According to the criteria of Landis and Koch (21), concordance was defined as poor, fair, moderate, good, and almost

Table 1. Demographic, clinical, and histopathologic features of the patients with systemic JIA–associated MAS and control patients*

Feature	Systemic JIA with MAS (n = 362)	Systemic JIA without MAS (n = 404)	Systemic infection (n = 345)	*P*†	*P*‡
Female	208 (57.5)	203 (50.2)	172 (49.9)	0.13	0.12
Age at onset of systemic JIA, median (IQR) years	5.3 (2.7–10.1)	5.5 (2.5–9.5)	–	0.80§	–
Age at onset of systemic infection, median (IQR) years	–	–	3.8 (1.5–8.6)	–	–
Fever	341/355 (96.1)	382/403 (94.8)	345 (100)	0.79	<0.0006
Hepatomegaly	245/350 (70)	123/400 (30.8)	39/344 (11.3)	<0.0001	<0.0001
Splenomegaly	201/347 (57.9)	95/399 (23.8)	23/344 (6.7)	<0.0001	<0.0001
Lymphadenopathy	178/346 (51.4)	115/396 (29)	29/344 (8.4)	<0.0001	<0.0001
Active arthritis	230/354 (65)	382/401 (95.3)	22 (6.4)	<0.0001	<0.0001
Central nervous system disease	122/349 (35)	7/400 (1.8)	34/344 (9.9)	<0.0001	<0.0001
Hemorrhagic manifestations	71/348 (20.4)	5/402 (1.2)	22/345 (6.4)	<0.0001	<0.0001
Bone marrow hemophagocytosis	149/249 (59.8)	–	–	–	–

* Except where indicated otherwise, values are the number of patients/total number with information available (%). IQR = interquartile range.
† Systemic juvenile idiopathic arthritis (JIA) with macrophage activation syndrome (MAS) versus systemic JIA without MAS, by chi-square test.
‡ Systemic JIA with MAS versus systemic infection, by chi-square test.
§ *P* value determined using the Mann-Whitney U test.

perfect for the kappa values of ≤0.20, 0.21–0.40, 0.41–0.60, 0.61–0.80, and 0.81–1, respectively.

Receiver operating characteristic (ROC) curve analyses were used to determine the cutoff value of each individual laboratory test for the differentiation between patients with MAS and control patients. Values that produced the most appropriate tradeoff between sensitivity and specificity were designated the cutoff values. For each cutoff, the sensitivity, specificity, area under the ROC curve (AUC) (with 95% confidence intervals [95% CIs]), and diagnostic odds ratio (DOR) (with 95% CIs) were calculated. The DOR of a test is the ratio of the odds of positivity in subjects with the disease to the odds of positivity in subjects without the disease; the value of the DOR ranges from 0 to infinity, with higher values indicating better performance of a discriminatory test (22). Values for laboratory biomarkers were not normalized, since neither the HLH-2004 guidelines nor the preliminary MAS guidelines require normalization of data.

RESULTS

The study sample included 362 patients with systemic JIA–associated MAS, 404 patients with active systemic JIA without MAS, and 345 patients with systemic infection, who were enrolled by 95 pediatric rheumatologists or hemato-oncologists practicing in 33 countries in 5 continents. The demographic data as well as the frequency of clinical features in patients with MAS and control patients are presented in Table 1. The proportion of female patients and the age at onset of systemic JIA were comparable between patients with MAS and patients with systemic JIA without MAS. All clinical manifestations were much more common in the MAS cohort than in both control groups, with the exception of fever, which was recorded in nearly all patients with MAS, nearly all patients with systemic JIA without MAS, and (as per the inclusion criterion) all patients with systemic infection, and with the exception of active arthritis, whose prevalence was higher in patients with systemic JIA without MAS than in patients with MAS. The prevalence of bone marrow hemophagocytosis could not be compared across groups because information about this finding was not available for patients with systemic JIA without MAS and for those with systemic infection.

Table 2 shows the findings from laboratory tests in the 3 patient groups. All laboratory values were more abnormal (showing either increased or decreased levels) in patients with MAS when compared with control patients, with the exception of the levels of serum sodium, which were comparable between patients with MAS and patients with systemic infection, and the erythrocyte sedimentation rate (ESR), which was comparable between patients with MAS and patients with systemic infection but was higher in patients with systemic JIA without MAS. Overall, the values from laboratory tests in patients with MAS were in line with expectations; that is, the findings reflected the typical changes that are known to occur in the syndrome.

The frequency of individual HLH-2004 criteria in

Table 2. Laboratory findings in the patients with systemic JIA–associated MAS and control patients*

Laboratory test	Systemic JIA with MAS (n = 362)	Systemic JIA without MAS (n = 404)	Systemic infection (n = 345)	P†	P‡
White blood cell count, $\times 10^9$/liter	9.9 (4.6–16.3)	16.8 (12.1–21.9)	12.2 (8–18.3)	<0.0001	<0.0001
Neutrophil count, $\times 10^9$/liter	5.4 (2.3–11.5)	11.9 (7.7–17.8)	6.8 (3.7–11.8)	<0.0001	0.002
Hemoglobin, gm/liter	9.8 (8.3–11.1)	10.1 (9.1–11.2)	11.8 (10.8–12.7)	0.019	<0.0001
Platelet count, $\times 10^9$/liter	144 (86–269)	498 (377–615)	340 (257–443)	<0.0001	<0.0001
Aspartate aminotransferase, units/liter	134 (58–338)	28 (20–39)	33 (25–44)	<0.0001	<0.0001
Alanine aminotransferase, units/liter	96 (37–234)	18 (11–34)	20 (13–32)	<0.0001	<0.0001
Lactate dehydrogenase, units/liter	1,203 (666–2,345)	438 (291–611)	507 (391–652)	<0.0001	<0.0001
Triglycerides, mg/dl	234 (151–318)	124 (91–142)	133 (100–192)	<0.0001	<0.0001
Albumin, gm/dl	3.1 (2.6–3.5)	3.5 (3–4)	3.8 (3.4–4.2)	<0.0001	<0.0001
Serum sodium, mmoles/liter	136 (132–138)	138 (135–140)	135 (133–138)	<0.0001	0.99
Fibrinogen, mg/dl	267 (152–437)	559 (463–720)	411 (293–559)	<0.0001	<0.0001
D-dimer, ng/ml	2,996 (1,094–7,550)	2,050 (501–4,064)	417 (135–972)	0.004	<0.0001
Ferritin, ng/ml	5,353 (1,500–13,040)	502 (158–1,627)	68 (33–133)	<0.0001	<0.0001
Erythrocyte sedimentation rate, mm/hour	48 (19–84)	78 (56–100)	40 (24–64)	<0.0001	0.47
C-reactive protein, mg/dl	9.2 (3.5–17.7)	8.9 (4.8–15.3)	3.8 (0.8–10)	0.99	<0.0001

* Values are the median (interquartile range). sJIA = systemic juvenile idiopathic arthritis; MAS = macrophage activation syndrome.
† Systemic juvenile idiopathic arthritis (JIA) with macrophage activation syndrome (MAS) versus systemic JIA without MAS, by Mann-Whitney U test.
‡ Systemic JIA with MAS versus systemic infection, by Mann-Whitney U test.

each patient sample is presented in Table 3. As expected, the presence of fever did not discriminate between the patient groups, since it was recorded in nearly all patients in each cohort. All of the other criteria were more common in patients with MAS than in control patients. However, the frequency of the criterion cytopenia in patients with MAS was very low (21.4%), owing to the stringent threshold value of blood cell counts. For the same reason, the criterion hypofibrinogenemia was met in only 24.8% of MAS cases. Although a ferritin level of ≥500 ng/ml was detected in ~90% of patients with MAS, it was also recorded in a sizeable percentage (50.4%) of patients with systemic JIA without MAS.

The frequency of individual items of the preliminary MAS guidelines in the 3 patient samples is presented in Table 4. The prevalence of all preliminary MAS criteria was much greater in patients with MAS than in each control group. Notably, in patients with MAS, a decreased platelet count and an increased aspartate aminotransferase level were much more frequent than were hypofibrinogenemia and leukopenia. Hepatomegaly was the most common clinical criterion

Table 3. Frequency of fulfillment of individual items of the HLH-2004 guidelines in the patients with systemic JIA–associated MAS and control patients*

Criterion	Systemic JIA with MAS (n = 362)	Systemic JIA without MAS (n = 404)	Systemic infection (n = 345)	P†	P‡
Fever	341/355 (96.1)	382/403 (94.8)	345 (100)	0.79	0.0006
Splenomegaly	201/347 (57.9)	95/399 (23.8)	23/344 (6.7)	<0.0001	<0.0001
Cytopenia (at least 2 of the 3 items)	64/299 (21.4)	2/264 (0.8)	9/337 (2.7)	<0.0001	<0.0001
Hemoglobin <90 gm/liter	120/335 (35.8)	84/389 (21.6)	21/343 (6.1)	<0.0001	<0.0001
Platelets <100 $\times 10^9$/liter	112/338 (33.1)	5/387 (1.3)	14/344 (4.1)	<0.0001	<0.0001
Neutrophils <1.0 $\times 10^9$/liter	25/297 (8.4)	2/267 (0.7)	8/340 (2.4)	0.0001	0.0017
Hypertriglyceridemia and/or hypofibrinogenemia	149/269 (62.8)	6/110 (5.5)	12/62 (19.4)	<0.0001	<0.0001
Triglycerides ≥265 mg/dl	113/278 (40.6)	5/137 (3.6)	12/96 (12.5)	<0.0001	<0.0001
Fibrinogen ≤1.5 gm/liter	73/294 (24.8)	2/213 (0.9)	1/138 (0)	<0.0001	<0.0001
Hemophagocytosis in bone marrow, spleen, or lymph nodes	160/252 (63.5)	–	–	–	–
Low or absent NK cell activity	–	–	–	–	–
Ferritin ≥500 ng/ml	277/308 (89.9)	136/270 (50.4)	7/209 (3.3)	<0.0001	<0.0001
Soluble CD25 ≥2,400 units/ml	–	–	–	–	–

* Values are the number of patients/total number with information available (%). HLH-2004 = 2004 diagnostic guidelines for hemophagocytic lymphohistiocytosis; NK = natural killer.
† Systemic juvenile idiopathic arthritis (JIA) with macrophage activation syndrome (MAS) versus systemic JIA without MAS, by chi-square test.
‡ Systemic JIA with MAS versus systemic infection, by chi-square test.

Table 4. Frequency of fulfillment of individual items of the preliminary MAS guidelines in the patients with systemic JIA–associated MAS and control patients*

Criterion	Systemic JIA with MAS (n = 362)	Systemic JIA without MAS (n = 404)	Systemic infection (n = 345)	P†	P‡
Platelet count ≤262 × 10^9/liter	251/338 (74.3)	30/387 (7.8)	3/344 (0.9)	<0.0001	<0.0001
Aspartate aminotransferase >59 units/liter	244/327 (74.6)	29/367 (7.9)	49/324 (15.1)	<0.0001	<0.0001
White blood cell count ≤4.0 × 10^9/liter	72/240 (30)	2/389 (0.51)	19/341 (5.6)	<0.0001	<0.0001
Fibrinogen ≤2.5 gm/liter	140/294 (47.6)	9/213 (4.2)	24/138 (17.4)	<0.0001	<0.0001
Central nervous system dysfunction	122/349 (35)	7/400 (1.8)	34/344 (9.9)	<0.0001	<0.0001
Hemorrhages	71/348 (20.4)	5/402 (1.2)	22 (6.4)	<0.0001	<0.0001
Hepatomegaly	245/350 (70)	123/400 (30.8)	39/344 (11.3)	<0.0001	<0.0001

* Values are the number of patients/total number with information available (%).
† Systemic juvenile idiopathic arthritis (JIA) with macrophage activation syndrome (MAS) versus systemic JIA without MAS, by chi-square test.
‡ Systemic JIA with MAS versus systemic infection, by chi-square test.

recorded in patients with MAS. CNS dysfunction and hemorrhages occurred in approximately one-third and one-fifth of the patients with MAS, respectively. The most common CNS manifestations were, in order of frequency, lethargy, seizures, irritability, confusion, headache, mood changes, and coma (results not shown).

The sensitivity, specificity, and kappa values for the adapted HLH-2004 guidelines and for the original and modified preliminary MAS guidelines in the differentiation of patients with MAS from control patients are presented in Table 5. The 3/5-adapted HLH-2004 guidelines revealed a better tradeoff between sensitivity and specificity and a higher kappa value than did the 4/5-adapted HLH-2004 guidelines in the discrimination of patients with MAS from patients with systemic JIA without MAS. The 4/5-adapted HLH-2004 guidelines had maximum specificity, but poor sensitivity. The best performance in distinguishing patients with MAS from patients with systemic JIA without MAS was provided by the original preliminary MAS guidelines, which showed both strong sensitivity and strong specificity, as well as the highest kappa value. The addition of the criterion of hyperferritinemia, defined at any threshold, to the preliminary MAS guidelines did not confer an appreciable advantage in terms of sensitivity, and led to decreased specificity and a decreased kappa value.

The 3/5-adapted HLH-2004 guidelines and the preliminary MAS guidelines modified with the addition of ferritin levels ≥500 ng/ml discriminated best between patients with MAS and patients with systemic infection. The 4/5-adapted HLH-2004 guidelines had maximum specificity, but poor sensitivity, whereas the original preliminary MAS guidelines were highly sensitive, but poorly specific.

Table 6 shows the cutoff values, calculated through the ROC curve analysis, as well as the sensitivity, specificity, AUC, and DOR of each individual laboratory test for the differentiation between patients with MAS and patients with systemic JIA without MAS. The best tradeoff between sensitivity and specificity was provided by the platelet count and the liver transaminase levels, whereas ferritin levels showed the greatest sensitivity, but only moderate specificity. The platelet count and levels of liver transaminases, lactate dehydrogenase,

Table 5. Sensitivity, specificity, and kappa values for the adapted HLH-2004 guidelines and the original and modified preliminary MAS guidelines in the discrimination of patients with systemic JIA–associated MAS from patients in each control group*

	Systemic JIA with MAS vs. systemic JIA without MAS			Systemic JIA with MAS vs. systemic infection		
	Sensitivity, %	Specificity, %	Kappa†	Sensitivity, %	Specificity, %	Kappa†
Adapted HLH-2004 guidelines (3 of 5 criteria)	79	75	0.53	79	98	0.74
Adapted HLH-2004 guidelines (4 of 5 criteria)	35	100	0.36	35	100	0.36
Preliminary MAS guidelines	86	86	0.71	95	29	0.28
Preliminary MAS guidelines + ferritin ≥500 ng/ml	90	50	0.41	86	95	0.76
Preliminary MAS guidelines + ferritin ≥1,000 ng/ml	84	63	0.48	81	97	0.71
Preliminary MAS guidelines + ferritin ≥2,000 ng/ml	69	78	0.46	66	98	0.54

* HLH-2004 = 2004 diagnostic guidelines for hemophagocytic lymphohistiocytosis; MAS = macrophage activation syndrome; JIA = juvenile idiopathic arthritis.
† Kappa values are Cohen's kappa statistics calculated for concordance with the diagnosis made by the attending physician.

Table 6. Best thresholds, sensitivity, specificity, AUC, and DOR values obtained for each laboratory feature in the assessment of discrimination between patients with systemic JIA–associated MAS and patients with systemic JIA without MAS*

Laboratory test	Best threshold	Sensitivity, %	Specificity, %	AUC (95% CI)	DOR (95% CI)
White blood cell count, $\times 10^9$/liter	≤10.55	55	82	0.72 (0.69–0.76)	5.6 (4–7.8)
Neutrophil count, $\times 10^9$/liter	≤4.33	45	94	0.74 (0.701–078)	13.8 (7.8–24.4)
Hemoglobin, gm/liter	≤8.1	25	93	0.56 (0.52–0.6)	4.2 (2.6–6.6)
Platelet count, $\times 10^9$/liter	≤271	75	92	0.83 (0.86–0.9)	35.3 (22.7–54.9)
Aspartate aminotransferase, units/liter	>60	74	93	0.88 (0.85–0.9)	36.4 (22.9–57.9)
Alanine aminotransferase, units/liter	>39	74	83	0.84 (0.81–0.87)	14.4 (9.8–21)
Lactate dehydrogenase, units/liter	>848	66	92	0.85 (0.81–0.88)	21.8 (13.2–36)
Triglycerides, mg/dl	>160	72	85	0.82 (0.78–0.86)	14.7 (8.6–25.3)
Albumin, gm/dl	<3.4	69	60	0.68 (0.64–0.72)	3.5 (2.4–4.9)
Serum sodium, mmoles/liter	≤135.9	51	74	0.66 (0.62–0.7)	2.9 (2–4.2)
Fibrinogen, mg/dl	<397	71	88	0.84 (0.81–0.87)	18 (11.1–29.1)
D-dimer, ng/ml	>5,900	32	88	0.62 (0.56–0.68)	3.6 (1.7–7.4)
Ferritin, ng/ml	>1,040	84	66	0.82 (0.78–0.85)	10.2 (6.9–15.2)
Erythrocyte sedimentation rate, mm/hour	≤39	46	89	0.7 (0.67–0.74)	6.9 (4.6–10.4)
C-reactive protein, mg/dl	≤2.3	20	92	0.51 (0.47–0.54)	2.6 (1.6–4.2)

* AUC = area under the curve; DOR = diagnostic odds ratio; JIA = juvenile idiopathic arthritis; MAS = macrophage activation syndrome; 95% CI = 95% confidence interval.

triglycerides, fibrinogen, and ferritin were the sole laboratory parameters that reached an AUC >0.80, and these parameters, together with the neutrophil count, had a DOR >10.

The laboratory test that discriminated best between patients with MAS and patients with systemic infection was the ferritin level, followed by levels of lactate dehydrogenase, levels of liver transaminases, the platelet count, and levels of albumin (results available from the corresponding author upon request). Notably, 4 patients with systemic infection were classified as having MAS by a panel of experts in the web-based consensus evaluations that preceded the consensus conference in which the new classification criteria for MAS in systemic JIA were developed (Ravelli A et al: unpublished observations).

DISCUSSION

We compared the validity of the HLH-2004 guidelines with the validity of the preliminary MAS guidelines as diagnostic tools for systemic JIA–associated MAS by analyzing their potential to discriminate between MAS and 2 conditions potentially confusable with MAS, represented by active systemic JIA without evidence of MAS and systemic infection. Both the MAS sample and the control samples were composed of large cohorts of patients recruited in large referral hospitals located in 33 countries in 5 continents. Of the 95 pediatric specialists who entered their patients' data, 83 were rheumatologists and 12 were hemato-oncologists. Notably, the hemato-oncologists only had data from patients with MAS. Owing to its size and sampling method, the study population is likely representative of patients with MAS and control illnesses seen in most tertiary care centers worldwide.

When we evaluated the ability of the guidelines to differentiate MAS from systemic JIA without MAS, we found that the preliminary MAS guidelines achieved the best tradeoff between sensitivity and specificity and the best concordance with the diagnosis made by the attending physician. The statistical performance of the 3/5-adapted HLH-2004 guidelines was overall inferior to that of the preliminary MAS guidelines, whereas the 4/5-adapted HLH-2004 guidelines revealed maximum specificity, but poor sensitivity. The addition of ferritin levels (at various thresholds) to the preliminary MAS guidelines did not increase appreciably the specificity and hampered the sensitivity. These results indicate that the preliminary MAS guidelines in their original format are best suited to diagnose MAS in patients with systemic JIA.

The poorer sensitivity of the HLH-2004 guidelines was mainly explained by the low frequency of 2 of the items, cytopenia (21.4%) and hypofibrinogenemia (24.8%), in patients with MAS. Failure to meet these criteria could be attributed to the uncommon occurrence of a platelet count, neutrophil count, and fibrinogen level below their respective thresholds of 100×10^9/liter, 1.0×10^9/liter, and 1.5 gm/liter. These findings may imply that most cases of MAS were diagnosed before the

drop in blood cell counts or drop in fibrinogen level below the levels required by the HLH-2004 guidelines. It is well known that patients with systemic JIA often have increased white blood cell and platelet counts as well as increased serum levels of fibrinogen as part of the underlying inflammatory process. Thus, the occurrence of a relative decline in these laboratory parameters, rather than the absolute decrease required by the HLH-2004 guidelines, may be more useful to make an early diagnosis of MAS. When patients with systemic JIA develop MAS, the degree of cytopenia and hypofibrinogenemia seen in HLH may only be reached in the late phase, when management may be more difficult. Recently, the HLH-2004 guidelines were found to have low sensitivity in the analysis of their ability to distinguish systemic JIA–associated MAS from familial and virus-associated HLH (23).

Although the stringency of the threshold levels for cytopenia and hypofibrinogenemia may enhance the specificity of the HLH-2004 guidelines, other items may be poorly specific. The criterion of fever did not discriminate between patients with MAS and control patients, since this feature was recorded in all or nearly all patients in each sample. Unfortunately, we could not obtain reliable information on the pattern of fever. However, it is common knowledge that the onset of MAS is often heralded by a shift from the high-spiking, intermittent pattern typical of active systemic JIA to a continuous, unremitting pattern (3–6). The criterion of ferritin levels ≥500 ng/ml was present in 89.9% patients with MAS, but also in 50.4% of patients with systemic JIA without MAS, which suggests that such a threshold level may not discriminate MAS from active systemic JIA. It is well known that many patients with active systemic JIA, in the absence of MAS, have ferritin levels above that threshold (24). In the acute phase of MAS, ferritin levels may peak to more than 5,000 ng/ml or even 10,000 ng/ml. Thus, a ferritin threshold greater than 500 ng/ml may be better suited to detect MAS in systemic JIA.

Hemophagocytosis was identified in 63.5% of MAS patients who had a bone marrow aspirate assessed or a reticuloendothelial organ biopsy performed. Considering that in 30.4% of cases, confirmation by tissue biopsy was not performed, the frequency of positive histopathologic findings of hemophagocytosis in the entire MAS sample was only 44.2%. Hemophagocytosis is known to be frequently absent in both HLH and MAS, particularly in their initial stages (25,26). Recently, isolated hemophagocytosis in marrow core biopsy specimens or aspirates was found to lack specificity for HLH (12). Notably, the demonstration of hemophagocytosis is not mandatory in either the HLH-2004 guidelines or the preliminary MAS guidelines.

The good diagnostic performance of the preliminary MAS guidelines was due to the strong ability of all individual criteria to discriminate between MAS and systemic JIA without MAS. However, among the laboratory criteria assessed, the items decreased platelet count and increased aspartate transaminase levels were recorded much more frequently among patients with MAS than were the items leukopenia and hypofibrinogenemia. This finding suggests that the latter 2 items may be less suitable for inclusion in a future revision of the diagnostic guidelines. As expected, among the clinical features observed, hepatomegaly was more common than CNS dysfunction and hemorrhages. However, liver enlargement was seen in 30.8% of patients with systemic JIA without MAS. Thus, because mild hepatomegaly is a frequent feature of active systemic JIA in the absence of MAS, this criterion should probably be reformulated as new-onset or worsening hepatomegaly. Surprisingly, the addition of hyperferritinemia (at various thresholds) to the preliminary MAS guidelines did not improve their diagnostic performance, owing to the drop in specificity. This phenomenon may be explained by the fact that many patients with systemic JIA have high ferritin levels in the absence of overt MAS or that the ferritin level varies among patients with MAS.

The 3/5-adapted HLH-2004 guidelines and the preliminary MAS guidelines modified with the addition of ferritin levels ≥500 ng/ml were the best discriminators between MAS and systemic infection. The 4/5-adapted HLH-2004 guidelines had maximum specificity, but poor sensitivity, whereas the original preliminary MAS guidelines were highly sensitive, but poorly specific. In the analysis of cutoff values for laboratory tests that provided the best discrimination between MAS and systemic JIA without MAS, the parameters that reached an AUC >0.80 were the platelet count, liver transaminase levels, lactic dehydrogenase levels, triglyceride levels, fibrinogen levels, and ferritin levels. These biomarkers may be the best candidates for inclusion among laboratory criteria in a future revision of the MAS guidelines. The cutoff for ferritin (1,040 ng/ml) revealed good sensitivity, but modest specificity. This implies that the criterion of ferritin levels alone may not be sufficient to detect MAS, because some patients with active systemic JIA without MAS may have a level above such a threshold. However, the application of a higher threshold may decrease sensitivity, that is, may lead to exclusion of some instances of MAS.

The laboratory parameters whose cutoff reached an AUC >0.80 in the discrimination between MAS and systemic infection were liver transaminase levels, lactate dehydrogenase levels, and ferritin levels. It is noteworthy that the discriminative potential of the ferritin level at a cutoff of 394.1 ng/ml was very strong, with both sensitivity and specificity being higher than 0.9 and the AUC being 0.97. In addition, only the MAS group as a whole demonstrated a ferritin level:ESR ratio of >80, a measure that has been suggested to distinguish MAS from new-onset systemic JIA flare (27).

Our analysis should be interpreted in light of some potential limitations. Patient data were collected through the retrospective review of clinical charts. A retrospective analysis is subject to the possibility of missing data and possibly erroneous data. Owing to the unavailability of data on bone marrow hemophagocytosis in control patients and the lack of data on NK cell activity and sCD25 levels in all patient samples, we could only apply adapted versions of the HLH-2004 guidelines, based on the application of only 5 of the 8 original criteria. Such an adaptation may have hampered the diagnostic performance of these criteria. Notably, the levels of sCD25 in the serum have been found to be a potential marker for subclinical MAS in patients with systemic JIA (6,28). However, NK cell activity or sCD25 assessments are not routinely performed, nor are they timely, in most pediatric rheumatology centers. Although all of the study cases were defined as MAS based on the clinician's expert opinion, some pediatric rheumatologists might have used the preliminary MAS guidelines as reference. This phenomenon may partially explain the better performance of these guidelines. We should also recognize the caveat that diagnostic categories of systemic JIA with MAS and that without MAS and febrile controls were determined by the reporting physician, which could introduce a bias. Furthermore, the accuracy of the diagnosis of MAS might have been affected by the level of experience of the physicians who participated in the study. However, the majority of the patients were diagnosed in tertiary care referring centers. Notably, the characteristics of patients with MAS enrolled by rheumatologists and those enrolled by hemato-oncologists were comparable (Minoia F et al: unpublished observations).

In conclusion, we found that the preliminary MAS guidelines possessed the strongest ability to identify MAS in the setting of systemic JIA. The addition of the item hyperferritinemia did not increase the sensitivity and specificity of the guidelines for MAS in systemic JIA, but enhanced their capacity to differentiate MAS from systemic infections. The diagnostic performance of the HLH-2004 guidelines should be further scrutinized in patient samples in which data on NK cell activity and sCD25 levels are available. However, the limited availability and lack of timeliness of these assays likely preclude their utility in identifying MAS in children with systemic JIA worldwide.

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