Dissecting the Heterogeneity of Macrophage Activation Syndrome Complicating Systemic Juvenile Idiopathic Arthritis

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ABSTRACT. ***Objective.*** To seek insights into the heterogeneity of macrophage activation syndrome (MAS) complicating systemic juvenile idiopathic arthritis (sJIA) through the analysis of a large patient sample collected in a multinational survey.

Methods. International pediatric rheumatologists and hemato-oncologists entered their patient data, collected retrospectively, in a Web-based database. The demographic, clinical, laboratory, histopathologic, therapeutic, and outcome data were analyzed in relation to (1) geographic location of caring hospital, (2) subspecialty of attending physician, (3) demonstration of hemophagocytosis, and (4) severity of clinical course.

Results. A total of 362 patients were included by 95 investigators from 33 countries. Demographic, clinical, laboratory, and histopathologic features were comparable among patients seen in diverse geographic areas or by different pediatric specialists. Patients seen in North America were given biologics more frequently. Patients entered by pediatric hemato-oncologists were treated more commonly with biologics and etoposide, whereas patients seen by pediatric rheumatologists more frequently received cyclosporine. Patients with demonstration of hemophagocytosis had shorter duration of sJIA at MAS onset, higher prevalence of hepatosplenomegaly, lower levels of platelets and fibrinogen, and were more frequently administered cyclosporine, intravenous immunoglobulin (IVIG), and etoposide. Patients with severe course were older, had longer duration of sJIA at MAS onset, had more full-blown clinical picture, and were more commonly given cyclosporine, IVIG, and etoposide.

Conclusion. The clinical spectrum of MAS is comparable across patients seen in different geographic settings or by diverse pediatric subspecialists. There was a disparity in the therapeutic choices among physicians that underscores the need to establish uniform therapeutic protocols. (J Rheumatol First Release April 15 2015; doi:10.3899/jrheum.141261)

Key Indexing Terms:
MACROPHAGE ACTIVATION SYNDROME SYSTEMIC JUVENILE IDIOPATHIC ARTHRITIS
HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS HEMOPHAGOCYTIC SYNDROMES

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Accepted for publication February 11, 2015.

The term macrophage activation syndrome (MAS) describes a hyperinflammatory complication of systemic juvenile idiopathic arthritis (sJIA) caused by severe hypercytokinemia because of a dysregulated immune response[1,2,3]. Cardinal signs and symptoms of MAS are unremitting fever, hepatosplenomegaly, neurologic dysfunction, and hemorrhagic manifestations. Typical laboratory abnormalities include pancytopenia and increased ferritin, triglycerides, liver enzymes, lactate dehydrogenase, D-dimers, and soluble CD25, and decreased fibrinogen and natural killer cell function. A characteristic histopathologic feature is the accumulation of macrophages exhibiting hemophagocytic activity in bone marrow core biopsy specimens or aspirates[3]. Although the estimated prevalence of MAS in sJIA is around 10%, reports suggest that the syndrome may occur subclinically in another 30–40% of cases[4,5]. Owing to its close similarity to the group of histiocytic disorders belonging to hemophagocytic lymphohistiocytosis (HLH), MAS is currently classified among the secondary, or acquired, forms of HLH[6,7].

If untreated, MAS may result in progressive multiorgan failure and eventual death. A timely diagnosis and the prompt initiation of life-saving treatment are therefore critical. However, early recognition of MAS is often challenging as there is no single pathognomonic clinical or laboratory variable. Further, hemophagocytosis may not be seen in the initial stages[8,9] and lacks specificity for hemophagocytic syndromes[10]. It is important to remember that MAS is not a diagnosis of exclusion and can complicate a wide variety of disorders, including sJIA[11]. In addition, the features of MAS may be hard to distinguish from those of conditions that may present with overlapping manifestations, such as flares of sJIA or systemic infections. The diagnostic difficulties are compounded by the variability in the severity potential of the syndrome, which may range from an acute, occasionally dramatic, presentation with full-blown clinical expression and rapid development of multiorgan failure to a more subtle course, characterized by only mild deterioration of general conditions and slight laboratory changes[12]. Recently, a wide disparity in the frequency and severity of the classical clinical and laboratory features across patients has been observed[13].

To increase the awareness and knowledge of MAS and facilitate its early diagnosis in daily practice, there is a need to better delineate its clinical spectrum through the evaluation of large patient samples. Further, to foster the achievement of a uniform approach to the management of the syndrome, it would be important to compare the therapeutic choices made by physicians practicing in different geographic areas or with different areas of expertise.

Against this background, the primary aim of our present study was to analyze the demographic, clinical, laboratory, histopathologic, therapeutic, and outcome data of 362 patients with sJIA-associated MAS enrolled in a multinational collaborative project[13,14,15] in relation to (1) the geographic location of the caring hospital, (2) the subspecialty of the attending physician, (3) the demonstration of hemophagocytosis in the bone marrow aspirate or reticuloendothelial organ biopsy, and (4) the severity of the clinical course.

MATERIALS AND METHODS

The study design, inclusion criteria, and data collection procedures have been described in detail previously[13,14]. Briefly, international pediatric rheumatologists and pediatric hemato-oncologists were invited by e-mail to participate in a data-collection study of patients with sJIA-associated MAS seen after 2002 with data recorded in their hospital's database. To be included in the study, patients had to have sJIA[16] and to have had an episode of MAS diagnosed and treated by the attending physician. In case the patients had multiple episodes of MAS, only the first episode had to be included. The study protocol was approved by the Institutional Review Board at each participating center. Investigators who participated in the study were asked to enter each patient's anonymous data into an electronic

Web-based database housed at the coordinating center (Gaslini Institute of Genoa).

Patient information included demographic data, triggering factors, clinical manifestations, laboratory variables, histopathologic features, therapeutic interventions, and outcome. Heart failure was defined as the occurrence of heart insufficiency or failure, heart decompensation, cardiac shock, or cardiac arrest. Lung failure was defined as the occurrence of respiratory or pulmonary failure or insufficiency, mechanical or artificial ventilation, or acute respiratory distress syndrome. Kidney failure was defined as the occurrence of renal or kidney insufficiency or failure or requirement for dialysis. Laboratory data were collected at 3 timepoints: (1) at last visit before onset of MAS, (2) at onset of MAS (defined as the time when the initial clinical and/or laboratory abnormalities suggesting the occurrence of MAS were detected), and (3) at full-blown MAS (defined as the time in which MAS reached the most acute stage). Except for blood counts and acute-phase reactants, values of laboratory tests were standardized based on the normal ranges provided by each local laboratory. All values were converted to the international standard unit system[17] as reported[13]. For the purpose of our present study, laboratory data collected at onset of MAS were used. The histopathologic section asked whether bone marrow aspirate or other biopsies were performed, and whether evidence of hemophagocytosis was detected. All medications administered for the treatment of MAS were to be recorded in the therapeutic section. Outcome assessment asked whether the patient was admitted to the intensive care unit (ICU) or had died.

Study comparisons. The demographic, clinical, laboratory, histopathologic, therapeutic, and outcome data of the study sample were analyzed by stratifying patients in relation to the following variables: (1) the continent in which the caring hospital was located (i.e., Europe vs North America vs other continents), (2) the subspecialty of the attending physician (i.e., pediatric rheumatology vs pediatric hemato-oncology), (3) the demonstration versus non-demonstration of hemophagocytosis in the bone marrow aspirate or reticuloendothelial organ biopsy, and (4) a severe versus a non-severe course. A severe course was defined as the need of admission to the ICU or a fatal outcome, whereas a non-severe course was defined as a course not complicated by any of these events.

Statistics. Quantitative data are presented as medians and interquartile ranges, and categorical data as absolute numbers and percentages. Quantitative and categorical data were compared by Mann-Whitney U test and chi-square test, respectively. To search for predictors of severity of the clinical course, multiple logistic regression analysis was performed, entering as explanatory variables the demographic, clinical, laboratory, histopathologic, and therapeutic features listed in Table 1 and Table 2, as well as the geographic location of the caring hospital and the subspecialty of the attending physician, with a severe course as the outcome variable. However, the neutrophil count was excluded, owing to its close correlation with the leukocyte count, and C-reactive protein, albumin, and D-dimer were excluded because of the excessive number of missing values. Before the application of logistic regression procedures, laboratory variables were dichotomized to binary variables by calculating their optimal cutoffs through receiver-operating characteristic (ROC) curve analysis. The backward elimination approach was chosen; this consisted of examining the effect of removing variables from the saturated model. The effect was expressed in terms of OR, and 95% CI were calculated; statistical significance was tested by likelihood ratio test. The area under the ROC of the best-fitting model was used as an indicator of the predictive ability of the model.

RESULTS

The data of the 362 patients with sJIA-associated MAS included in our present study were entered in the Web-based database by 95 investigators from 33 countries. Of the 362 patients, 179 (49.4%) were enrolled in Europe, 72 (19.9%) in North America, and 111 (30.7%) in other continents. Two hundred eighty-three patients (88.2%) were included by pediatric rheumatologists and 79 patients (21.8%) by pediatric hemato-oncologists. The proportion of patients entered by pediatric hemato-oncologists was greater in North America than in Europe and other continents (37.5%, 25.1%, and 6.3%, respectively, $p < 0.0001$). A bone marrow aspirate or lymphoreticular organ biopsy was performed in 252 of 348 patients (72.4%) for whom this information was available: 159 (63.1%) exhibited macrophage hemophagocytosis, whereas 93 (36.9%) did not. Histopathologic confirmation of hemophagocytosis by tissue biopsy was not looked for in 96 patients. Thus, hemophagocytosis was detected in 159/348 patients (45.7%) and was not found or looked for in 189/348 patients (54.3%). Of the 252 patients who had a bone marrow aspirate or lymphoreticular organ biopsy performed, 15 (5.9%) had undergone a lymph node biopsy, of whom only 4 were positive for hemophagocytosis. A severe course was reported in 92 of 347 patients (26.5%) for whom this information was available. The demographic and clinical features of the entire patient sample, as well as the data on triggers and therapeutic interventions, were reported previously[13].

The comparison of patient characteristics by geographic location of the caring hospital is presented in Table 1. Patients followed in Europe had a lower frequency of central nervous system (CNS) disease and higher levels of aspartate aminotransferase (AST) than patients seen in North America or in other continents, whereas hemoglobin levels were lower in patients seen in other continents compared to Europe and North America. North American physicians used intravenous immunoglobulin (IVIG) and other biologic medications more frequently than physicians from Europe and other continents. Outcome was overall worse in patients followed in other continents.

The comparison of patient data in relation to the specialty of the attending physician showed that patients entered by pediatric hemato-oncologists had a greater frequency of heart, lung, or kidney failure (23.4% vs 6.1%, $p < 0.0001$) and were treated more commonly with biologics (23.7% vs 12.8%, $p = 0.02$) and etoposide (18.4% vs 9.9%, $p = 0.04$), whereas patients seen by pediatric rheumatologists received cyclosporine more frequently (67.3% vs 39.5%, $p < 0.0001$). All typical clinical and laboratory features of MAS were comparable between patients seen by the 2 groups of pediatric specialists (results not shown).

The comparison of features between patients who were found to have hemophagocytosis on bone marrow aspirate or lymphoreticular organ biopsy ($n = 159$) and patients who had hemophagocytosis not detected or looked for ($n = 189$) revealed that patients with hemophagocytosis had shorter duration of sJIA at MAS onset (0.1 vs 0.5 yr, $p = 0.024$), greater prevalence of hepatomegaly (78.7% vs 63.4%, $p = 0.002$) and splenomegaly (68.0% vs 50.8%, $p = 0.001$), lower levels of platelets (131 vs 171 $\times 10^9$/l, $p = 0.007$) and fibrinogen (237 vs 282 mg/dl, $p = 0.022$), higher levels of triglycerides (243 vs 228 mg/dl, $p = 0.033$), and were more

Table 1. Comparison of demographic, clinical, laboratory, therapeutic, and outcome data of patients with MAS by geographic location of the caring hospital. Values are n (%) or median (IQR) unless otherwise specified.

Characteristic	n	Europe, n = 179	n	North America, n = 72	n	Other Continents, n = 111	p
Demographic features at onset of MAS							
Age, yrs	169	7.8 (3.9–13.7)	71	8.8 (4.2–13.1)	105	7.9 (4.2–11.7)	0.78
Duration of sJIA, yrs	168	0.2 (0.04–2.4)	71	0.3 (0.1–3.0)	105	0.8 (0.1–2.6)	0.05
Clinical manifestations at onset of MAS							
Fever	174	167 (96.0)	71	68 (95.8)	110	106 (96.4)	0.98
Lymphadenopathy	173	86 (50.0)	66	39 (59.1)	108	53 (49.1)	0.38
Hepatomegaly	173	122 (70.5)	70	46 (65.7)	107	77 (72.0)	0.66
Splenomegaly	173	96 (55.5)	67	34 (50.8)	107	61 (66.4)	0.08
Hemorrhagic manifestations	172	32 (18.6)	69	13 (18.8)	107	26 (24.3)	0.49
CNS involvement	174	49 (28.2)	68	29 (42.7)	107	44 (41.1)	0.03
Arthritis	175	105 (60.0)	71	51 (71.8)	108	74 (68.5)	0.14
Heart, lung, or kidney failure	175	16 (9.1)	70	11 (15.7)	109	8 (7.34)	0.17
Laboratory tests at onset of MAS							
Hemoglobin, g/dl	155	9.9 (8.5–11.2)	68	10.2 (8.9–11.6)	104	9.4 (7.6–11.0)	0.04
White blood cell count, × 10^9/l	160	9.2 (4.8–15.6)	70	10.3 (5.8–16.3)	104	10.1 (4.1–18.1)	0.77
Neutrophil count, × 10^9/l	144	4.6 (2.2–9.6)	55	6.4 (2.7–11.8)	98	5.6 (2.1–13.2)	0.62
Platelet count, × 10^9/l	164	139 (79–248)	70	170 (88–382)	104	148 (90–257)	0.31
ESR, mm/h	141	50 (19–84)	64	55 (19–88)	83	38 (18–76)	0.59
CRP, mg/dl	158	9.8 (3.8–18.2)	59	7.7 (3.3–16.9)	78	9.3 (2.1–17.6)	0.43
AST, U/l	162	142 (70–317)	67	82 (46–158)	98	102 (37–350)	0.01
LDH, U/l	145	386 (256–733)	53	329 (196–859)	71	488 (223–1133)	0.43
Triglycerides, mg/dl	135	228 (162–328)	54	244 (170–355)	89	266 (161–390)	0.20
Albumin, g/dl	111	3.6 (3.0–4.0)	60	3.3 (2.8–3.9)	87	3.6 (3.1–4.1)	0.11
Fibrinogen, mg/dl	143	245 (140–361)	66	222 (163–379)	85	184 (116–375)	0.18
Ferritin, ng/ml	152	8072 (2095–19,395)	69	8368 (2285–25,407)	87	8727 (1233–21,433)	0.51
D-dimer, ng/ml	75	3510 (1467–7580)	60	2784 (1472–8161)	40	3851 (1042–7912)	0.86
Histopathologic features							
Patients who underwent BM aspirate or biopsy of lymphoreticular organs	172	129 (75.0)	69	46 (66.7)	107	77 (72.0)	0.42
Patients with hemophagocytosis	129	82 (63.6)	46	28 (60.9)	77	49 (63.6)	0.94
Therapy							
Any corticosteroids	173	167 (96.5)	69	67 (97.1)	107	107 (100.0)	0.16
Cyclosporine	173	100 (57.8)	68	45 (66.2)	107	68 (63.6)	0.41
IVIG	172	44 (25.6)	65	35 (53.9)	107	46 (43.0)	< 0.0001
Biologics	172	23 (13.4)	64	22 (34.4)	105	7 (6.7)	< 0.0001
Etoposide	172	25 (14.5)	63	4 (6.4)	104	11 (10.6)	0.20
Outcome							
ICU admission	119	32 (26.9)	47	17 (36.2)	69	33 (47.8)	0.01
Death	173	7 (4.1)	69	5 (7.3)	105	16 (15.2)	0.004

MAS: macrophage activation syndrome; IQR: interquartile range; sJIA: systemic juvenile idiopathic arthritis; CNS: central nervous system; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; BM: bone marrow; IVIG: intravenous immunoglobulin; ICU: intensive care unit.

frequently administered cyclosporine (74.4% vs 50.0%, p < 0.0001), IVIG (44.9% vs 29.4%, p = 0.003), and etoposide (18.8% vs 6.0%, p = 0.0003). Outcomes were similar in patients with or without hemophagocytosis. When the comparison was restricted to the 252 patients who had undergone histopathologic assessment, the only significant differences between patients with (n = 159) or without (n = 93) hemophagocytosis were a higher frequency of splenomegaly (68.0% vs 55.0%, p = 0.041), and a greater use of cyclosporine (74.2% vs 44.6%, p < 0.0001) and IVIG (44.9% vs 28.3%, p = 0.01) in patients with hemophagocytosis.

Table 2 shows the comparison of characteristics between patients who experienced a severe course and patients who had a non-severe course. As compared with patients with a non-severe course, patients with a severe course were older and had longer duration of sJIA at MAS onset; greater frequency of hepatomegaly, hemorrhagic manifestations, CNS dysfunction, and heart, lung, or kidney failure; lower levels of erythrocyte sedimentation rate (ESR), albumin, and fibrinogen; higher levels of AST, lactate dehydrogenase, triglycerides, and D-dimer; and were more commonly given cyclosporine, IVIG, and etoposide.

For the multivariate analysis of predictors of severity of the clinical course, complete data were available on 123 patients. Independent correlations with a severe course were identified for CNS involvement (OR 72.3, 95% CI

Table 2. Comparison of demographic, clinical, laboratory, therapeutic, and outcome data of patients with MAS by severity of clinical course. Values are n (%) or median (IQR) unless otherwise specified.

Characteristic	n	Patients with Severe Course, n = 92	n	Patients without Severe Course, n = 255	p
Demographic features at onset of MAS					
Age, yrs	88	9.0 (4.9–14.0)	248	7.7 (3.6–12.2)	0.03
Duration of sJIA, yrs	88	1.0 (0.1–4.1)	247	0.2 (0.05–2.3)	0.006
Clinical manifestations at onset of MAS					
Fever	90	88 (97.8)	254	243 (95.7)	0.53
Lymphadenopathy	87	43 (49.4)	249	130 (52.2)	0.65
Hepatomegaly	88	70 (79.6)	252	168 (66.7)	0.02
Splenomegaly	87	54 (62.1)	250	142 (58.8)	0.39
Hemorrhagic manifestations	89	33 (37.1)	249	34 (13.7)	< 0.0001
CNS involvement	89	59 (66.3)	250	59 (23.6)	< 0.0001
Arthritis	90	56 (62.2)	254	166 (65.4)	0.59
Heart, lung, or kidney failure	91	26 (28.6)	253	8 (3.2)	< 0.0001
Laboratory tests at onset of MAS					
Hemoglobin, g/dl	87	9.5 (7.8–10.8)	241	9.8 (8.4–11.2)	0.27
White blood cell count, × 10^9/l	86	10.1 (4.8–14.6)	241	9.9 (4.6–17.1)	0.61
Neutrophil count, × 10^9/l	74	5.4 (2.7–9.9)	218	5.3 (2.1–11.6)	0.60
Platelet count, × 10^9/l	86	129 (81–216)	245	163 (89–296)	0.05
ESR, mm/h	69	30 (15–72)	213	52 (20–85)	0.03
CRP, mg/dl	71	10.2 (2.9–17.6)	217	8.5 (3.7–17.7)	0.87
AST, U/l	85	187 (48–499)	233	111 (53–228)	0.03
LDH, U/l	67	584 (283–1494)	198	360 (225–690)	0.006
Triglycerides, mg/dl	76	275 (180–392)	196	237 (163–331)	0.04
Albumin, g/dl	71	2.9 (2.8–3.8)	183	3.7 (3.1–4.1)	0.002
Fibrinogen, mg/dl	77	166 (85–302)	212	249 (157–378)	< 0.0001
Ferritin, ng/ml	71	11,448 (1862–35,967)	230	8169 (1787–17,641)	0.08
D-dimer, ng/ml	45	5511 (2445–10,465)	126	2645 (1159–6478)	0.002
Histopathologic features					
Patients who underwent BM aspirate or biopsy of lymphoreticular organs	92	64 (69.6)	254	186 (73.2)	0.50
Patients with hemophagocytosis	64	43 (67.2)	186	114 (61.3)	0.40
Therapy					
Any corticosteroids	92	92 (100.0)	255	247 (96.9)	0.12
Cyclosporine	92	67 (72.8)	254	145 (57.1)	0.008
IVIG	91	52 (57.1)	251	71 (28.2)	< 0.0001
Biologics	91	19 (20.9)	248	33 (13.3)	0.08
Etoposide	90	20 (22.2)	247	19 (7.7)	0.0002

MAS: macrophage activation syndrome; IQR: interquartile range; sJIA: systemic juvenile idiopathic arthritis; CNS: central nervous system; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; BM: bone marrow; IVIG: intravenous immunoglobulin.

13.6–383.7); heart, lung, or kidney failure (OR 49.0, 95% CI 5.5–432.8); hemoglobin ≤ 7.9 g/dl (OR 6.5, 95% CI 1.5–28.8); and age at onset of MAS > 11.5 years (OR 6.4, 95% CI 1.7–24.6).

DISCUSSION

We sought insights into the clinical heterogeneity of sJIA-associated MAS by analyzing the data of 362 patients seen by 95 pediatric subspecialists, either rheumatologists or hemato-oncologists, practicing in 33 countries in 5 continents. Owing to its size and sampling method, the study population is likely representative of patients with MAS seen in most tertiary care centers worldwide and covers the entire spectrum of disease phenotype and severity.

Our results show that the demographic, clinical, laboratory, and histopathologic features of sJIA-associated MAS are overall comparable among patients seen in different geographic areas, although European patients may be less likely to experience CNS involvement and more vulnerable to liver dysfunction. There was a disparity in the use of some medications, with more frequent administration of IVIG and biologics in North America and less use of biologics in other continents. The lack of availability of biologic agents (e.g., anakinra), which may have an important therapeutic role in severe or refractory cases[18,19,20,21], may be partly responsible for the greater mortality rate reported in patients followed in hospitals located in the other continents group, which included several developing countries. In addition, a correlation with ICU admission and mortality has previously been suggested[22].

That patient features were comparable across patients seen by rheumatologists and hemato-oncologists is reassuring because rheumatologists might have been more eager to base the diagnosis on the preliminary diagnostic guidelines for sJIA-associated MAS[23] whereas hemato-oncologists might have been more likely to refer to the 2004 diagnostic guidelines for HLH[24]. The greater prevalence of heart, lung, or kidney failure among patients seen by hemato-oncologists is difficult to explain, given the similarity of all the other features. However, it may reflect a tendency toward a greater severity of multiorgan involvement among patients referred to hemato-oncologists. The disparity in the prevalence of heart, lung, or kidney failure between specialists does not depend on differences in the definitions of these items, which were the same for all investigators who entered their patients' data. Not unexpectedly, rheumatologists more frequently used cyclosporine, whose therapeutic role in sJIA-associated MAS has been particularly emphasized in the rheumatology literature[25,26], whereas hemato-oncologists revealed a greater preference for etoposide, which is a central medication in HLH therapeutic protocols[27,28]. Biologic medications were also administered more commonly by hemato-oncologists. This observation may explain, at least in part, the greater frequency of biologics use among patients seen in North America, given the greater proportion of patients entered by hemato-oncologists in North America as compared with Europe and other continents.

The shorter duration of sJIA at MAS onset among patients with demonstration of hemophagocytosis may reflect either a greater probability of detection of this morphologic finding in patients who developed MAS at the beginning of the underlying disease or the tendency by the caring physicians to preferentially perform a bone marrow aspirate or a reticuloendothelial organ biopsy in patients who have possible or probable sJIA (i.e., who lack arthritis) and were candidates for this procedure in the context of the initial diagnostic investigations. The greater frequency of hepatosplenomegaly and the lower platelet count in patients with hemophagocytosis may be an indirect confirmation that histopathologic confirmation was looked for more commonly in cases of diagnostic uncertainty with a hematologic malignancy. Patients with hemophagocytosis might have been regarded as having a more serious disease by their physicians because they were given cyclosporine, IVIG, and etoposide more frequently than patients without hemophagocytosis. However, most of the above differences were no longer detectable when the comparison between patients with or without hemophagocytosis was restricted to the patients who had undergone a tissue biopsy.

That the clinical and laboratory picture and course of MAS were comparable between patients who had or did not have the demonstration of hemophagocytosis is in keeping with the view that tissue confirmation of hemophagocytosis may not be essential for the diagnosis of MAS. Hemophagocytosis is known to be frequently absent in both HLH and MAS, particularly in their initial stages[8,9]. In addition, isolated hemophagocytosis in marrow core biopsy specimens or aspirates was found to lack specificity for HLH[10]. Notably, the demonstration of hemophagocytosis is not mandatory in either the HLH-2004 diagnostic guidelines[24] or the preliminary MAS diagnostic guidelines[23]. The latter guidelines, with or without the addition of hyperferritinemia, were recently found to have strong diagnostic sensitivity for MAS complicating sJIA[15]. Thus, because it is critical that MAS be diagnosed early, to start a timely proper treatment, further delay by performing bone marrow aspirate or organ biopsies may not be justified or necessary in many patients.

That patients with severe course had a longer duration of sJIA at MAS onset suggests that the prognosis of the syndrome may be more guarded when it occurs in established as opposed to early sJIA. However, patients with severe course had a more full-blown clinical picture with a greater frequency of the major clinical manifestations (e.g., CNS involvement, liver failure, coagulopathy) and more pronounced abnormality of most laboratory biomarkers, particularly those related to liver involvement. The lower ESR among those with severe course likely reflects decreased fibrinogen from consumptive coagulopathy. Multivariate analysis revealed that patients at higher risk for a severe course were those who had CNS dysfunction, heart, lung, or kidney failure, lower level of hemoglobin, and were older at MAS onset. Altogether, these observations are in keeping with the common clinical experience that the sickest patients are at greater risk of requiring ICU admission or having a fatal course. As expected, patients with severe course were treated more aggressively, as shown by the greater use of cyclosporine, IVIG, and etoposide. The prevalence of hemophagocytosis was comparable between patients with or without severe course, which implies that this finding may not have prognostic implication. Indeed, an animal model of MAS suggested that the presence of hemophagocytosis was solely dependent on the relative absence of interleukin 10[28].

Our results should be interpreted in the light of some potential limitations. Patient data were collected through the retrospective review of clinical charts, which is subject to missing and possibly erroneous data. Further, because the participating centers were not asked specifically to include all consecutive patients, we cannot exclude a selection bias in patient enrollment. During the time frame of patient inclusion (2002–2012), there have been some variations in the treatment approach to MAS, with patients seen in the earlier years being more likely to have received corticosteroids with or without cyclosporine and those treated in recent years being more likely to have received biologic medications or etoposide. Further, the publication of diagnostic guidelines for HLH in 2004[24] and for sJIA-associated MAS in 2005[23] may have facilitated earlier and more accurate diagnosis in recent years. We should also

recognize the caveat that all the study cases were defined as MAS based on clinician expert opinion. However, in the absence of validated diagnostic criteria for the syndrome, there was no better way to ascertain the accuracy of the diagnosis of sJIA and MAS.

The main strengths of our study were the size of the patient sample, which is the largest ever studied (to our knowledge), and the comprehensiveness of the data collection.

Our findings show that the clinical spectrum of MAS is comparable across patients seen in different geographic settings or by pediatric specialists with different areas of expertise. Hemophagocytosis was found more commonly in patients who developed MAS near the onset of sJIA, but its demonstration did not have prognostic implication. The sickest patients were at greatest risk for a poorer outcome. There was a disparity in the therapeutic choices among caring physicians, which underscores the need of clinical trials or consensus initiatives aimed to define uniform therapeutic protocols for the syndrome.

ACKNOWLEDGMENT

The authors thank the following physicians who contributed to the study by including their patients' data: Mario Abinun, MD (Newcastle, UK); Amita Aggarwal, MD (Lucknow, India); Maria Alessio, MD (Naples, Italy); Jordi Anton, MD (Barcelona, Spain); Maria Teresa Apaz, MD (Cordoba, Argentina); Itziar Astigarraga, MD (Bilbao, Spain); Tadej Avcin, MD (Ljubljana, Slovenia); Vyacheslav Chasnyk, MD (St. Petersburg, Russian Federation); Rolando Cimaz, MD (Florence, Italy); Fabrizia Corona, MD (Milan, Italy); Ruben Cuttica, MD (Buenos Aires, Argentina); Gianfranco D'Angelo, MD (Ancona, Italy); Eli Eisenstein, MD (Jerusalem, Israel); Graciela Espada, MD (Buenos Aires, Argentina); Michel Fischbach, MD (Muenster, Germany); Michael Frosch, MD (Muenster, Germany); Maria Luz Gamir, MD (Madrid, Spain); Yi-Jin Gao, MD (Shanghai, China); Alexei Grom, MD (Cincinnati, Ohio, USA); Soad Hashad, MD (Tripoli, Libya); Zeng Huasong, MD (Guangzhou, China); Adam Huber, MD (Halifax, Nova Scotia, Canada); Norman Ilowite, MD (New York, New York, USA); Antonella Insalaco, MD (Rome, Italy); Marijia Jelusic-Drazic, MD (Zagreb, Croatia); Ozgur Kasapcopur, MD (Istanbul, Turkey); Raju Khubchandani, MD (Mumbai, India); Toshiyuki Kitoh, MD (Nagakute, Japan); Sheila Knupp Feitosa de Oliveira, MD (Rio de Janeiro, Brazil); Isabelle Kone-Paut, MD (Paris, France); Kai Lehmberg, MD (Hamburg, Germany); Loredana Lepore, MD (Trieste, Italy); Caifeng Li, MD (Beijing, China); Despoina Maritsi, MD (Athens, Greece); Deborah McCurdy, MD (Orange, California, USA); Rosa Merino, MD (Madrid, Spain); Paivi Miettunen, MD (Calgary, Alberta, Canada); Velma Mulaosmanovic, MD (Sarajevo, Bosnia and Herzegovina); Susan Nielsen, MD (Copenhagen, Denmark); Seza Ozen, MD (Ankara, Turkey); Priyankar Pal, MD (Kolkata, India); Sampath Prahalad, MD (Salt Lake City, Utah, USA); Donato Rigante, MD (Rome, Italy); Ricardo Russo, MD (Buenos Aires, Argentina); Helga Sanner, MD (Oslo, Norway); Sujata Sawhney, MD (New Delhi, India); Bita Shakoory, MD (Philadelphia, Pennsylvania, USA); Susan Shenoi, MD (Seattle, Washington, USA); Artur Silva Clovis, MD (Sao Paulo, Brazil); Valda Stanevicha, MD (Riga, Latvia); Gary Sterba, MD (Caracas, Venezuela); Gordana Susic, MD (Belgrade, Serbia); Flavio Sztajnbok, MD (Rio de Janeiro, Brazil); Syuji Takei, MD (Kagoshima City, Japan); Ralf Trauzeddel, MD (Berlin, Germany); Elena Tsitsami, MD (Athens, Greece); Erbil Unsal, MD (Izmir, Turkey); Yosef Uziel, MD (Kfar Saba, Israel); Jennifer Weiss, MD (Hackensack, New Jersey, USA); Carol A. Wallace (Seattle, Washington, USA); Sheila Weitzman, MD (Toronto, Ontario, Canada); Carine Wouters, MD (Leuven, Belgium); Nico Wulffraat, MD (Utrecht, Netherlands); Mabruka Zletni, MD (Tripoli, Libya).

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