Occult Macrophage Activation Syndrome in Patients with Systemic Juvenile Idiopathic Arthritis

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ABSTRACT. ***Objective.*** Macrophage activation syndrome (MAS) is a well described, but purportedly uncommon manifestation of systemic juvenile idiopathic arthritis (SJIA). There is evidence to suggest that macrophage activation is integral to the pathogenesis of SJIA. Accordingly, many patients with SJIA may have evidence of mild MAS that is not appreciated clinically. We investigated the prevalence of occult MAS in children with SJIA by reviewing bone marrow aspirates (BMA).

Methods. Patients diagnosed with SJIA who underwent bone marrow aspiration were identified retrospectively. Patients admitted with a diagnosis of fever of unknown origin and discharged with a diagnosis other than SJIA or malignancy, and who had a BMA, were identified as controls. The BMA were reviewed by a single hematopathologist for evidence of MAS, ranging from activated macrophages to frank hemophagocytic cells.

Results. Eight of 15 (53%) patients with SJIA had BMA suggestive of MAS. Two of 15 patients (13%) were diagnosed clinically with MAS. Three patients (20%) were noted to have frank hemophagocytosis, only one of whom was diagnosed with MAS clinically. There were no statistically significant differences in the laboratory values for the patients with and without evidence of MAS on BMA. There was no evidence of increased macrophage activity or hemophagocytosis in any of the control BMA.

Conclusion. Occult MAS appears to be common in patients with SJIA who undergo BMA. This suggests that macrophage activation may be integral to the pathogenesis of SJIA, with implications for treatment. (First Release Mar 1 2007; J Rheumatol 2007;34:1133–8)

Key Indexing Terms:

MACROPHAGE ACTIVATION SYNDROME
HEMOPHAGOCYTOSIS
BONE MARROW ASPIRATE
SYSTEMIC JUVENILE IDIOPATHIC ARTHRITIS
CD163 ANTIGEN
HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

Macrophage activation syndrome (MAS) is the terminology used to describe the secondary hemophagocytic lymphohistiocytosis (HLH) associated with systemic juvenile idiopathic arthritis (SJIA). The clinical picture of MAS consists of fever, hepatosplenomegaly, lymphadenopathy, cytopenia, coagulopathy, and central nervous system inflammation. Other symptoms may include rash (usually fixed), serositis, and myocarditis with possible progression to renal and respiratory failure. It is often life-threatening, and occasionally fatal. Characteristic laboratory changes include elevated D-dimers, ferritin, liver enzymes, and prothrombin time, in conjunction with falling fibrinogen and cell counts. MAS is reported to occur in about 7% of patients with SJIA[1]. The pathologic hallmark of MAS is the presence of hemophagocytosis, histiocytes engulfing hematopoetic cells, in the bone marrow aspirate (BMA). HLH in general is thought to be the result of an ineffectual cytotoxic response directed against the activated macrophages. Patients with genetic deficits in the effector cytotoxic granule pathway, including perforin and MUNC-13-4, develop primary HLH, with features indistinguishable from MAS. The inability to effectively lyse and kill infected macrophages leads to an unending cycle of activation between CD8 T lymphocytes and macrophages. The result is the overproduction of cytokines, such as interferon-γ from T cells[2,3], and interleukin 1ß from macrophages[4]. It is thought that this hypercytokinemia is responsible at least in part for the pathology associated with HLH[5].

Children with active SJIA share a number of clinical features that are similar to the presentation of MAS; indeed, it is sometimes difficult to distinguish between MAS and a flare of the underlying disease. The symptoms of what is classified as active SJIA may actually be part of a spectrum of the disease process of MAS in terms of their severity. The International League of Associations for Rheumatology criteria[6] for the diagnosis of SJIA include fever (usually > 103.6°F), hepatosplenomegaly, and lymphadenopathy, all of which are also hallmarks of MAS. It has been observed that patients

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with SJIA also have mildly elevated D-dimers[7], prothrombin times[8], and ferritin levels[9]. Thus, current approaches to identifying MAS[10] may not be sensitive enough. Further, children with SJIA have natural killer cell and perforin defects[11], a finding associated with both primary and secondary HLH, even in the absence of MAS.

Given the large degree of overlap between SJIA and MAS, we hypothesize that active SJIA and MAS may be a single entity within a spectrum of severity, with fulminant MAS being the most severe form. Thus, clinical MAS, defined by falling cell counts and erythrocyte sedimentation rate (ESR), organomegaly, hemophagocytosis, and accompanying laboratory changes as outlined above, may represent the most severe manifestations of active SJIA. As a corollary to this, patients with more mild disease activity may have an "occult MAS" that is currently underappreciated. As a result, we predicted that the BMA of patients with active SJIA would show a greater frequency of hemophagocytosis than expected from the clinical presentation of MAS[1].

MATERIALS AND METHODS

Patients diagnosed with SJIA (ICD-9 code 714.30) and who underwent BMA between January 1, 1998, and January 1, 2005, were identified within the hospital database. In all cases, the decision to obtain a bone marrow biopsy was at the discretion of a pediatric rheumatologist. Most often, this was done to rule out hematologic malignancy. In a few instances, there was clinical suspicion of MAS at the time of biopsy. Patients who were admitted with a diagnosis of fever of unknown origin (ICD-9 code 780.6) and discharged with a diagnosis other than SJIA or malignancy and who underwent BMA were identified as controls (N = 4). Patients diagnosed during the same time period with SJIA who did not undergo BMA served as additional controls. All aspirates were re-reviewed by a single hematopathologist (MP), blinded to the original reports, and were categorized into the following 4 groups: normal, activated macrophages, mild hemophagocytosis, or frank hemophagocytosis. Activated macrophages were defined by the presence of cytoplasmic vacuolization and prominent nucleoli. Aspirates with > 1 hemophagocytic cell per half-slide were considered positive for mild hemophagocytosis. BMA with > 1 hemophagocytic cell per high-power field were considered to demonstrate frank hemophagocytosis. Available laboratory and clinical data were obtained retrospectively and recorded on a standard form for all patients with SJIA. The clinical diagnosis of MAS was confirmed if this diagnosis was documented in the medical record by the attending pediatric rheumatologist at the time of the patient's illness. The clinical criteria that led to a clinical diagnosis of MAS were usually falling or low cell counts, low or falling ESR, organomegaly, and hemophagocytosis present on bone marrow examination. "Occult MAS" was defined by the presence of activated macrophages or hemophagocytosis in the BMA of patients without the clinical diagnosis of MAS. Differences in laboratory and clinical variables between those with occult MAS and those without were tested for statistical significance ($p < 0.05$) using Fisher's exact and Wilcoxon rank-sum tests, where appropriate, using Stata 9.0 software (Stata, College Station, TX, USA). These same comparisons were made for patients with SJIA who underwent BMA and those that did not. In addition to bone marrow aspirates, bone marrow biopsies were available for 3 patients with SJIA and 2 controls. There were no clear indications why bone marrow biopsies were performed for certain patients. The biopsies were stained for CD163 (clone 10D6; Vector Laboratories, Burlingame, CA, USA), a marker for histiocytes, and were reviewed by MP, who was blinded to diagnosis and aspirate results for degree of staining (scored on a scale from 1 to 4) and highlighting of hemophagocytic cells. CD163 staining was performed only on biopsy core specimens as aspirates were not suitable for CD163 staining. Institutional review board permission was obtained.

RESULTS

Sixteen patients with SJIA who underwent BMA were identified. Clinical characteristics of these patients between time of presentation and the performance of BMA are shown in Table 1. Twenty-nine patients diagnosed with SJIA during the study period did not undergo BMA. However, the 16 patients identified in this study did not appear to be exceptional in their presentation, most having the typical quotidian fever, evanescent rash, or synovitis expected in SJIA. Patients that underwent BMA had lower hemoglobins (median 9.0 g/dl vs 10.3 g/dl; $p = 0.02$, Wilcoxon rank-sum test) and had more lymphadenopathy ($p = 0.005$, Wilcoxon rank-sum test) than those who did not undergo BMA. There were no statistically significant differences in white blood cell (WBC) count, platelets, ESR, ferritin, or age. Further, there were no statistical differences in the presence of arthritis, rash, hepatomegaly, or splenomegaly between the 2 groups. Thus, the 16 patients identified for this study were similar to the population of SJIA patients seen as a whole over the study period.

One BMA was unavailable for review (Patient 4). Of the 15 patients available for analysis, only 2 (13%) had the clinical diagnosis of MAS as documented in the chart (Table 2). The diagnoses were made on the basis of falling cell counts and BMA results. Patient 12 had an abrupt drop in all 3 blood cell lines and Patient 14 had an abrupt drop in 2 cell lines with elevated liver transaminases. Because these abrupt falls in cell counts are characteristic of clinical MAS, diagnostically they can be as important as, if not more important than, the absolute values of the cell counts themselves.

Upon review, 8 (53%) patients had evidence of occult MAS on their BMA (Table 2). Further, 3 patients were found to have frank hemophagocytosis on the BMA, and only one of these 3 patients had the clinical diagnosis of MAS. The other 2 patients did not exhibit any clinical signs of MAS, including low platelets, elevated AST, low WBC, low fibrinogen, central nervous system dysfunction, hemorrhages, or hepatomegaly[10]. By comparison, none of the BMA control cases had evidence of activated macrophages or hemophagocytosis. A formal analysis of the differences in clinical and laboratory features between patients with and those without occult MAS was inhibited by small sample size and missing data. However, all 5 patients with moderate or severe lymphadenopathy and both patients with splenomegaly had occult MAS. Among patients in whom the attending physician did not make a clinical diagnosis of MAS, elevated D-dimers (> 1.0) were found in 4 of 5 and markedly elevated ferritins (> 1000) were found in 2 of 3 patients.

CD163 staining of the bone marrow biopsies was markedly increased in all SJIA patients examined (Table 2, Figure 1A). The stain highlighted hemophagocytosis in all 3 SJIA patients, even in one case where it was not evident on the hematoxylin and eosin stain. Both control bone marrows showed much less staining intensity for CD163, and did not highlight excess hemophagocytosis (Table 2, Figure 1B).

Table 1. Patient characteristics.

Patient	Age at BMA, yrs	Duration of Symptoms Prior to BMA, wks	Active Synovitis	HSM	Lymph-adenopathy	Pericardial Effusion
1	10	3	No	None	Mild	No
2	4	3	Yes	None	Moderate	No
3	7	5	Yes	Moderate	Moderate	No
4	4	2	No	None	Mild	No
5	7	10	Yes	None	None	No
6	1	5	Yes	None	Mild	No
7	2	21	Yes	Mild (liver only)	None	No
8	8	4	Yes	None	Mild	No
9	3	4	No	None	None	No
10	4	3	No	None	Mild	No
11	1	3	Yes	None	None	No
12	3	2	No	Mild	Moderate	Yes
13	2	26	Yes	None	Severe	No
14	9	8	Yes	None	None	No
15	12	10	Yes	None	Moderate	No
16	7	3	No	None	Mild	No

BMA: Bone marrow aspiration, HSM: hepatosplenomegaly.

Table 2. Laboratory and bone marrow aspirate findings.

Patient	WBC	Hb	Plt	ESR	CRP	D-dimer	Fibrinogen	Ferritin	Triglyceride	PT	Initial BMA	BMA Review	CD163
1	11.6	9.0	393,000	83	10.4	NA	NA	NA	NA	13.2	Normal	Normal	NA
2	39.6	9.6	716,000	77	28.9	NA	NA	1421	NA	NA	Normal	HPC	NA
3	17.5	7.8	461,000	98	43.08	NA	497	NA	NA	13.5	Normal	HPC	NA
4	12.8	10.3	576,000	96	NA	NA	NA	NA	NA	NA	Normal	Unavailable	NA
5	7.5	11.7	440,000	38	4.25	NA	NA	NA	47	13.2	Normal	Normal	NA
6	17.7	7.5	756,000	80	18.7	NA	428	NA	97	12.2	Normal	Normal	NA
7	8.9	8.0	613,000	72	8.12	NA	NA	NA	NA	NA	Normal	Normal	NA
8	14.4	9.9	365,000	92	4.19	6.36	NA	NA	98	12.9	Normal	Normal	NA
9	19.8	8.3	774,000	50	7.7	2.39	419	NA	NA	12.8	Normal	Normal	NA
10	20.0	11.5	364,000	15	5.51	NA	NA	NA	188	NA	Normal	Normal	NA
11	9.9	8.5	576,000	NA	4.3	10.22	530	51	NA	13.5	Activated macro	Activated macro	NA
12*	22.8	7.9	217,000	135	15.5	5.47	427	NA	NA	12.7	Frank HPC	Frank HPC	NA
13	7.9	7.2	290,000	56	NA	NA	NA	NA	NA	NA	Normal	Activated macro	++++/HPC
14*	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Normal	HPC	++++/HPC
15	16.3	9.5	307,000	89	12.5	10.29	568	8810	207	13.4	Frank HPC	Frank HPC	NA
16	7.4	10.1	435,000	90	4.6	1.00	609	NA	NA	NA	Frank HPC	Frank HPC	++++/HPC
Control 1	5.7	9.7	458,000	131	3.0	NA	NA	NA	NA	NA	Normal	Normal	++
Control 2	9.3	9.2	257,000	80	4.8	NA	NA	NA	NA	NA	Normal	Normal	+
Control 3	6.3	9.6	392,000	56	4.6	14.9	NA	968	NA	13.8	Normal	Normal	NA
Control 4	5.9	12.6	293,000	43	4.6	0.86	449	NA	NA	NA	Normal	Normal	NA

* Patient with clinical diagnosis of macrophage activation syndrome. WBC: white blood cell count normal range 4.5–13.0 thousand cells/μl, Hb: hemoglobin normal range 12.0–16.0 g/dl, Plt: platelets normal range 150,000–400,000 cells/μl, ESR: erythrocyte sedimentation rate normal range 0–20 mm/h, CRP: C-reactive peptide normal range < 1.0 mg/dl, D-dimer normal range 0.1–0.6 μg/ml fibrinogen equivalent units, fibrinogen normal range 172–471 mg/dl, triglyceride (nonfasting) normal range 37–130 mg/dl, PT: prothrombin time normal range 11.0–13.5 s, BMA: bone marrow aspirate, macro: macrophages, HPC: hemophagocytosis, NA: not available.

DISCUSSION

MAS can be a severe, even fatal, complication of SJIA. In this study, over one-half of patients with SJIA we reviewed had elements in their bone marrow suggestive of MAS. This is roughly 7 times more often than the reported prevalence of 7% for MAS in SJIA[1]. The frequency of the clinical diagnosis of MAS (13%) in our series was similar to the reported prevalence. Interestingly, 2 of the 3 patients in whom a ferritin

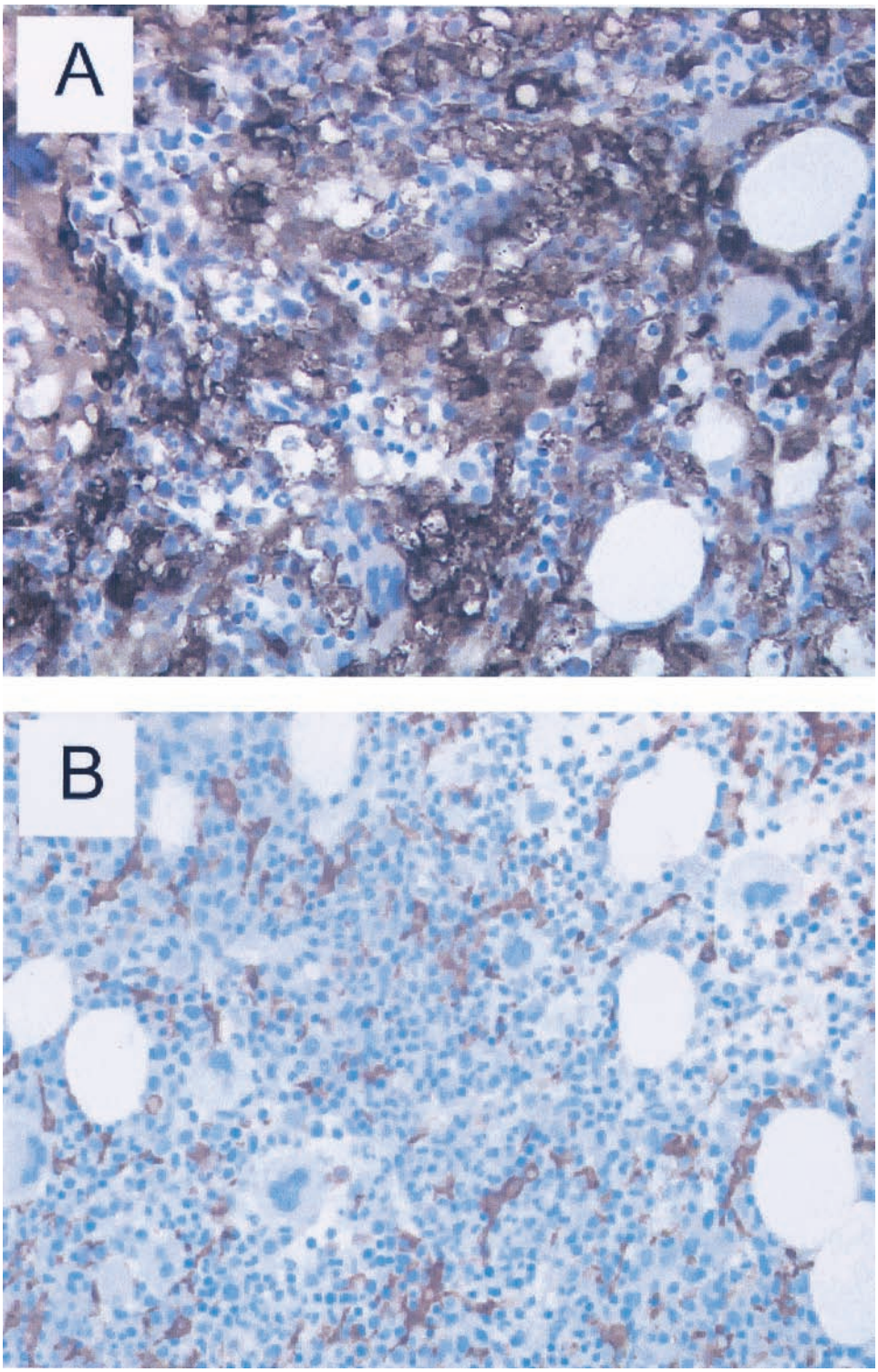

Figure 1. CD163-stained bone marrow biopsies of a patient with SJIA (A) and a control patient (B) (400× magnification).

level was measured had elevated values despite not having other features diagnostic of MAS. The finding of activated macrophages and hemophagocytosis is not a normal finding in the marrow of healthy patients[12], and we saw no evidence of these findings in the BMA of febrile control patients. Engulfment of cells may not be seen in hemophagocytic syndromes, but rather, a proliferation and morphologic activation of histiocytes may be seen[13]. We found many BMA that

showed such histiocytic features. Not all the SJIA patients during the study period had a BMA, so it is possible there is some selection bias in our sample. However, the only differences noted between our sample and the patients with SJIA who did not undergo BMA were slightly lower hemoglobin and increased lymphadenopathy. Therefore we believe these patients are reasonably representative of the disease process in general. Regardless of any differences in hemoglobin or lymphadenopathy, the frequency of MAS in children with active SJIA may be notably higher than previously reported.

Similarly, Min, *et al* reported that 4 of 12 patients (33%) with adult onset Still's disease, a clinical entity similar to SJIA, had evidence of either activated macrophages or hemophagocytosis on BMA[14]. None of these patients had clinical evidence of MAS either. The question thus remains whether MAS is present to some degree in all SJIA patients with active disease, and that we simply do not have sensitive enough methods or criteria to establish the diagnosis. Along this line, the high frequency of histiocytosis with hemophagocytosis in the bone marrows of patients with SJIA suggests that this pathologic feature may be integral to the disease itself, rather than being a rare complication. MAS has a clinical and laboratory overlap with SJIA. Many of our patients with SJIA had hepatosplenomegaly and lymphadenopathy. In addition, we noted significantly elevated ferritin levels in 2 patients with SJIA that did not have clinical evidence of MAS. Many of these patients also have elevated D-dimers, a finding normally associated with MAS. These observations are consistent with the idea that SJIA may represent a subclinical, "occult" MAS, and that patients who develop the fulminant form of MAS are manifesting the most severe end of the spectrum of the disease. This notion has also been proposed by Ramanan, *et al*, also based on the association of elevated ferritin and D-dimer values[15]. These results suggest that we need a formal definition of MAS that accounts for these "occult" cases. Biochemical markers of macrophage activation may provide assistance in formulating such a definition.

CD163, a haptoglobin scavenger receptor, is a marker specific for macrophages. CD163 used as an immunohistochemical marker for histiocytes is more specific than CD68 and has been used for evaluation of hemophagocytosis in previous reports[16]. Excess staining in spleen as well as increased serum soluble CD163 has been reported in MAS[16]. Recent investigations suggest that CD163-positive macrophages are particularly important in hemophagocytosis associated with sepsis. The erythrophagocytosis that may be mediated in part by the CD163 haptoglobin receptor may provide a substrate for the production of heme oxygenase-1 (HO-1). HO-1 in turn is protective in sepsis by limiting an overexuberant inflammatory response[17]. Thus, these macrophages may in fact be attempting to limit the uncontrolled inflammation seen in MAS. CD163 immunostaining on bone marrow biopsies allows direct visualization of the number of macrophages and their cytoplasmic contents. Cells that may not be recognized as being engulfed by macrophages may be highlighted by a rim of CD163 staining, demonstrating that they are actually within the cytoplasm of a macrophage. Although normal bone marrows may have some low levels of basal, physiologic CD163-positive staining, similar to the control marrows in this study (1+/2+), the marrows of children with active SJIA showed much more markedly abnormal staining (4+), despite only one of these patients being clinically diagnosed with MAS (Figure 1A). Interestingly, 2 of these patients with abnormal CD163 stains had an initial BMA read as normal, including the one that was clinically diagnosed with MAS. CD163 immunostaining revealed the occult macrophage activation and hemophagocytosis in both these cases, which was not seen in the control bone marrow biopsies available for staining. We propose that abnormal CD163 immunostaining may be a useful diagnostic tool for the diagnosis of SJIA in the absence of other causes for MAS. Measurement of soluble CD163 in the serum of patients may be a less invasive means of obtaining similar information[16]. Early detection of MAS may allow for early aggressive treatment of the underlying disease in order to prevent fulminant MAS.

Our findings suggest that MAS may more ubiquitously accompany SJIA than was previously recognized. Accordingly, we propose that SJIA and MAS represent a spectrum of the same entity, ranging from its most severe, life-threatening form of fulminant MAS to its most mild, occult form manifest only by biochemical abnormalities and bone marrow hemophagocytosis. Given our more recent understanding of the antiinflammatory role of CD163-positive, hemophagocytosing macrophages, it is possible that these cells may be a compensatory reaction against a systemic inflammatory state caused by the fundamental genetic and/or environmental triggers for SJIA/MAS. Investigation into the genetics of SJIA, and what factors predispose patients to enter the severe MAS end of the spectrum rather than remain in the occult form, may help to clarify these issues.

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