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Efficacy of Moderately Dosed Etoposide in Macrophage Activation Syndrome–Hemophagocytic Lymphohistiocytosis

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ABSTRACT. ***Objective.*** Macrophage activation syndrome (MAS) constitutes 1 subtype of the hyperinflammatory syndrome hemophagocytic lymphohistiocytosis (HLH), and the term MAS-HLH was recently proposed for HLH with underlying autoimmune/autoinflammatory conditions. The mortality of MAS-HLH has been estimated at 5–10%. Here we report our experiences with moderately dosed etoposide in severe MAS-HLH; the objective was to effectively reduce severe hyperinflammatory activity with limited side effects.

Methods. In addition to conventional antiinflammatory treatment, moderately dosed etoposide was administered to 7 children affected by rapidly progressing MAS-HLH with central nervous system (n = 5) and/or pulmonary (n = 5) involvement. Three had underlying systemic juvenile idiopathic arthritis (sJIA), 2 had atypical sJIA (no arthritis at diagnosis), and 2 had systemic lupus erythematosus. We performed lymphocyte cytotoxicity analyses in all 7 and genetic analyses in 6.

Results. All children promptly responded to moderately dosed etoposide (50–100 mg/m^2 once weekly), added to conventional MAS-HLH treatment that was considered insufficient. The mean accumulated etoposide dose was 671 mg/m^2 (range 300–1050 mg/m^2) as compared to 1500 mg/m^2 recommended in the first 8 weeks of the HLH-94/HLH-2004 protocols. One child developed neutropenic fever and another neutropenic sepsis (neutrophils 0.3 × 10^9/L at therapy onset). Five of 7 children had low percentages (< 5%) of circulating natural killer (NK) cells prior to or in association with diagnosis; NK cell activity was pathologically low in 2 of 5 children studied. Disease-causing variants in HLH-associated genes were not found. All children were alive at latest follow-up (2–9 yrs after onset); neurological symptoms had normalized in 4 of 5 affected children.

Conclusion. Moderately dosed etoposide may be beneficial in severe and/or refractory MAS-HLH.

Key Indexing Terms: etoposide, hemophagocytic lymphohistiocytosis, juvenile idiopathic arthritis, macrophage activation syndrome, systemic lupus erythematosus

Macrophage activation syndrome (MAS) is a life-threatening hyperinflammatory syndrome with poor prognosis. The mortality has been estimated at around 8%.[1] New treatment approaches are therefore warranted.

MAS constitutes 1 subtype of the hyperinflammatory syndrome hemophagocytic lymphohistiocytosis (HLH), characterized by fever, hyperferritinemia, cytopenia, and coagulopathy, which may progress to multiorgan failure.[2] HLH includes primary (familial/genetic) and secondary (acquired) HLH (sHLH). HLH in patients with underlying autoimmune or

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autoinflammatory conditions has historically been called MAS. More recently, the term MAS-HLH was proposed.[2]

In primary HLH, survival increased remarkably from 0% to approximately 60% in the international studies HLH-94 and HLH-2004.[3,4] These successful chemo-immunotherapy regimens are built on etoposide and dexamethasone, with additional cyclosporine A (CSA), followed by allogeneic hematopoietic stem cell transplant (HSCT) for primary HLH. Dosages and frequency of etoposide in these intensive protocols are associated with a considerable risk of neutropenia and infections, whereas the risk for secondary leukemia is very limited (0.3–0.4%).[3,4] The addition of etoposide dramatically improved survival in other forms of HLH and is recommended in both infection- and malignancy-associated HLH.[5,6,7]

For MAS-HLH, most treating physicians use high-dose corticosteroids and CSA.[1] Further, treatment with the interleukin (IL)-1 inhibitor anakinra is rapidly expanding,[8] and many patients respond to an increased dosage of anakinra.[9,10] In refractory severe adult MAS, etoposide has been reported to be very effective. Nevertheless, reports on etoposide in pediatric MAS-HLH are limited.

We present 7 children with MAS-HLH for whom rapid deterioration and central nervous system (CNS) and/or pulmonary involvement prompted us to administer etoposide in addition to conventional antiinflammatory treatment. To reduce the risk of severe neutropenia, and since sHLH/MAS-HLH typically requires less aggressive cytotoxic therapy, we used reduced etoposide dosages compared to the HLH-94/HLH-2004 protocols.[3,4] Moreover, genetic and lymphocyte cytotoxicity analyses were performed.

METHODS

We report 7 children with MAS-HLH (3 systemic juvenile idiopathic arthritis [sJIA], 2 atypical sJIA, and 2 systemic lupus erythematosus [SLE]), aged 0.4–16 (median 9) years at MAS-HLH onset, treated with etoposide during 2010–2017. Four children were at Karolinska University Hospital, Stockholm, 2 were at Sahlgrenska University Hospital, Gothenburg, and 1 was at Umeå University Hospital; the last 3 were in collaboration with Karolinska University Hospital. Clinical and laboratory data were retrieved from the patients' medical records.

The sJIA diagnosis was made according to the International League of Associations for Rheumatology criteria. Two sJIA patients were labeled as "atypical," as they did not have arthritis at diagnosis. Both patients with SLE fulfilled the 1997 American College of Rheumatology revised SLE criteria. All fulfilled the diagnostic guidelines for MAS for children with sJIA and SLE, respectively; 4 also fulfilled the HLH-2004 diagnostic criteria.[3]

The ethics committee of the Karolinska Institutet, Stockholm, approved the studies (2006/228-31/3, 2010/1596-31/4, 2013/1723-31/4). Informed consent was obtained for all patients. For methods on lymphocyte cytotoxicity assays and genetic analyses, see Supplementary Methods (available with the online version of this article).

RESULTS

Severity of MAS-HLH. All children were severely ill and had CNS involvement or did not respond to conventional MAS-HLH therapy; 6 required intensive care unit support (Table 1). Three had previous MAS-HLH episodes. Five had CNS involvement during the MAS-HLH episodes (moderate/severe = 2, very severe = 3; Table 1). Patient 1 displayed neurological symptoms with confusion and cognitive impairment, magnetic resonance imaging (MRI) showing CNS inflammation, and electroencephalogram (EEG) indicating encephalitis. Patient 7 had 2 episodes of posterior reversible encephalopathy syndrome. CNS involvement in Patients 2, 3, and 5 presented with initial disorientation with rapid deterioration of cerebral function, followed by seizures and finally coma; EEG and CNS MRI were pathological in all 3 showing extensive encephalopathy and encephalitis.

Severe lung disease requiring mechanical ventilation developed in Patients 4, 6 and 7, initially with subtle respiratory signs and symptoms, but a persistent cough and hypoxia ensued, leading to respiratory distress. In Patient 4, bronchoalveolar lavage revealed pulmonary alveolar proteinosis (PAP) confirmed by lung biopsy. The severe lung disease progressed even after the acute MAS-HLH episodes had subsided. In Patient 6, pulmonary symptoms worsened in conjunction with MAS-HLH reoccurrence, concurrent with influenza A, and the patient was then diagnosed with pulmonary arterial hypertension (PAH). Patient 7 displayed voluminous yellow airway secretions; computed tomography (CT) scan showed diffuse lung infiltration and pleural effusions.

MAS-HLH treatments. All initially received intravenous (IV) methylprednisolone (MP) pulse therapy followed by oral steroids. Additionally, CSA was given to 2 and anakinra to 3 (up to 2.7–15 mg/kg/d; Table 2). Nevertheless, severe MAS-HLH activity remained and, therefore, IV etoposide was initiated. For clinical variables and laboratory values at etoposide initiation, see Table 1.

Etoposide doses ranged between 50–100 mg/m^2 once weekly (Table 2). However, Patient 2, with severe Epstein-Barr virus (EBV) infection, received 150 mg/m^2 once weekly from Week 4 and, additionally, rituximab (RTX; Table 2). Patient 6 had 3 separate etoposide-treated MAS-HLH episodes. The total number of etoposide doses administered to all patients varied between 4 and 11. Cumulative etoposide doses ranged between 300–1050 mg/m^2 (median 800 mg/m^2, mean 671 mg/m^2), as compared to 1500 mg/m^2 during the 8-week initial therapy in HLH-94/HLH-2004 protocols[3,4] (Table 2).

Clinical outcomes. All children responded very well to dose-reduced etoposide treatment. The effect is illustrated by the rapidly decreasing ferritin levels in Figure 1.

At a median follow-up of 6 years, all patients were alive with full pulmonary recovery. Six had no neurological late effects, but Patient 5 still had severe neurological impairment. Patient 6 suffered recurring MAS-HLH episodes and underwent allogeneic HSCT followed by 95% autologous reconstitution but without disease reactivation.

Toxicity and safety. The medical records were reviewed for toxicity (Table 2). Severe etoposide-associated toxicity affected Patient 4 (neutropenic septicemia after first etoposide dose; neutrophils 0.3 × 10^9/L at start). Patient 2 developed neutropenic fever.

Lymphocyte cytotoxicity and genetic analyses. Five of 7 children had low percentages (< 5%) of circulating NK cells in peripheral

Table 1. Clinical and laboratory findings in 7 patients with MAS-HLH immediately prior to etoposide treatment (after first-line therapy).

Patient	1	2	3	4	5	6[a]	7
Sex	Male	Female	Female	Male	Female	Female	Female
Parental consanguinity	No	No	No	No	No	No	No
Age at onset of MAS-HLH, yrs	16	9	3	5	15	5 months	16
Underlying disease	SLE	sJIA	sJIA	sJIA	SLE	Atypical sJIA	Atypical sJIA
Time from disease onset to MAS-HLH, months	6	60	10	3	1	5	7
Previous treatment	Oral steroids, HCQ	Oral steroids, MP pulses, ETN	ETN, MP pulses	Oral steroids, IVIG, CSA, anakinra, MP pulses	Oral steroids, HCQ	Anakinra, TCZ, MP pulses	Oral steroids, anakinra
Ongoing treatment at the time of MAS-HLH diagnosis	Oral steroids, HCQ	TCZ, MTX	Oral steroids, TCZ, MTX	Oral steroids, CSA, anakinra	Oral steroids, HCQ	Oral steroids, anakinra, CSA	Oral steroids, anakinra
Infection	None identified	EBV	VZV	None identified	UTI: *E. coli*	None identified	None identified
MAS criteria for sJIA[26]	N/A	Yes	Yes	Yes	N/A	Yes	Yes
MAS criteria SLE[27]	Yes	N/A	N/A	N/A	Yes	N/A	N/A
HLH 2004 criteria (fulfilled/evaluated)	5/8	6/8	5/8	3/8	6/7	4/6	4/6
Fever	No	Yes	Yes	Yes	Yes	Yes	Yes
Splenomegaly	Yes	Yes	Yes	No	No	Yes	Yes
Bicytopenia	Yes	Yes	No	No	Yes	No	No
Hemoglobin (< 90 g/L)	79	88	104	94	85	93	91
Neutrophils (< 1.0 × 10^9/L)	3.3	6.4	3.2	0.3	0.4	11.8	7.9
Platelets (< 100 × 10^9/L)	45	29	54	121	150	84	180
Triglycerides (> 3.0 mmol/L)	4.0	6.1	9.1	2.0	8.6	4.5	4.6
or fibrinogen (< 1.5 g/L)	1.7	0.5	0.9	1.6	1.4	1.8	4.7
Hemophagocytosis in BM	No	No	No	No	ND	No	No
Ferritin (> 500 mg/L)	20,778	121,937	25,946	36,007	12,558	5025	15,000
sCD25 levels (> 2400 U/mL)	2172	> 7500	> 7500	3309	3460	ND	ND
NK cell activity defective[b]	Yes	No	No	No	Yes	ND	ND
CNS involvement	Yes	Yes	Yes	No	Yes	No	Yes
Neurological manifestations	Moderate	Very severe	Very severe	No	Very severe	No	Moderate
Abnormal CNS MRI	Yes	Yes	Yes	No	Yes	No	Yes
EEG findings	Encephalitis	Encephalitis	Encephalitis	Not done	Encephalitis	Not done	Not done
Known pulmonary involvement	No	No	Yes	Yes	Yes	Yes	Yes
Recurring MAS-HLH	No	No	No	Yes	No	Yes	Yes

[a] Patient 6 had a total of 3 episodes with MAS (1 triggered by influenza A), each of which required a course of etoposide. [b] In first sample analyzed; NK cell activity defective < 10 lytic units. BM: bone marrow; CNS: central nervous system; CSA: cyclosporine A; EBV: Epstein-Barr virus; EEG: electroencephalogram; ETN: etanercept; HCQ: hydroxychloroquine; IVIG: intravenous immunoglobulins; MAS: macrophage activating syndrome; MP: methylprednisolone; MRI: magnetic resonance imaging; MTX: methotrexate (subcutaneous); N/A: not applicable; ND: not determined; NK: natural killer; sCD25: soluble interleukin-2 receptor; sJIA: systemic juvenile idiopathic arthritis; SLE: systemic lupus erythematosus: TCZ: tocilizumab; UTI: urinary tract infection; VZV: varicella-zoster virus.

blood mononuclear cells prior to or in association with diagnosis, of which 3 also had low absolute NK cell numbers (< 0.07 × 10^9/L). In 2 children with NK lymphopenia, NK cell activity was pathologically low (≤ 10 lytic units) and NK cell degranulation defective (< 5%; Supplementary Methods and Supplementary Table 1, available with the online version of this article).

Genetic analyses were performed in 6 patients. Selected HLH-associated genes were analyzed in 4 patients, whereas whole genome sequencing was performed in 2 patients. No suspected pathogenic variants were identified in HLH-associated genes in any of the patients (Supplementary Methods and Supplementary Table 1, available with the online version of this article).

Patient descriptions. The clinical course and treatment in Patients 1–3 and 5–7 are presented below. Patient 4 is presented in Figure 1. Of note, the duration of the etoposide treatment in many of these patients is longer than we currently would recommend from acquired knowledge.

- *Patient 1.* A previously healthy 16-year-old boy with a 4-month history of SLE developed accelerating inflammatory disease (ferritin 20,778 μ/L), but while his laboratory values improved markedly following MP pulses, his CNS remained affected with moderate confusion and cognitive impairment. Because MRI confirmed CNS inflammation and an EEG indicated encephalitis, additional treatment with etoposide 75 mg/m^2 weekly was initiated. Subsequently, his CNS symptoms disappeared within 6 days, and he recovered fully; a subsequent MRI was normal.
- *Patient 2.* A 9-year-old girl with sJIA on treatment with tocilizumab (TCZ) and oral methotrexate (MTX) was

Table 2. Treatment and long-term outcome of 7 patients with MAS-HLH treated with etoposide.

Patient	1	2	3	4	5	6	7
First-line therapy for MAS-HLH	MP pulses	MP pulses	MP pulses	MP pulses, anakinra (4 mg/kg)	MP pulses	MP pulses, anakinra (15 mg/kg)	MP pulses, anakinra (2.7 mg/kg)
ICU care	No (evaluated by ICU)	Yes	Yes	Yes	Yes	Yes	Yes
Complications	Severe hyponatremia, lupus nephritis, pericardial effusion	No	Hypertonia	Kidney stones, hydronephrosis	No	No	No
Etoposide	75 mg/m^2 × 4	100 mg/m^2 × 3 150 mg/m^2 × 5	100 mg/m^2 × 9	50 mg/m^2 × 2 100 mg/m^2 × 7	50 mg/m^2 × 3 75 mg/m^2 × 2 100 mg/m^2 × 2	50–75 mg/m^2 × 4[a] 75 mg/m^2 × 5[a] 75 mg/m^2 × 2[a]	50 mg/m^2 × 7
Weeks on etoposide[b]	4	8.5	8	9.5	6	4+6+2[a]	8
Accumulated etoposide dose, mg/m^2	300	1050	900	800	500	800	350
Toxicities during etoposide therapy							
Neutrophils < 0.5 × 10^9 (days)	0	4	2	18	6	0	0
Platelets < 50 × 10^9 (days)	0	8	0	0	2	0	0
Platelets < 20 × 10^9 (days)	0	1	0	0	0	0	0
Neutropenic fever and/or severe infections	0	Neutropenic fever[c]	0	Neutropenic sepsis[d]	0	0	0
Infection prophylaxis	Oral fungal	PCP, fungal	PCP, fungal	PCP, fungal	PCP, fungal	PCP, fungal	PCP, fungal
Additional MAS-HLH treatment	Oral steroids	Oral steroids, CSA, rituximab	Oral steroids	Oral steroids, CSA	Oral steroids, CSA, plasmapheresis	Oral steroids, CSA, IVIG, anakinra	Oral steroids, IVIG
Treatment after MAS-HLH	HCQ	None	Anakinra, TCZ	6-mercaptopurine, CSA	Azathioprine, HCQ	None	TCZ, CSA
HSCT	No	No	No	No	No	Allogeneic HSCT	No
Outcome and follow-up	Alive 9 yrs after onset	Alive 6 yrs after onset	Alive 8 yrs after onset	Alive 8 yrs after onset	Alive 6 yrs after onset	Alive 2 yrs after HSCT	Alive 2 yrs after onset
Treatment at last follow-up	HCQ	None	None	None	HCQ	None	TCZ
Clinical response	Complete	Complete	Complete	Complete	Severe CNS sequelae	Complete[e]	Complete

[a] Patient 6 had a total of 3 episodes with MAS (1 triggered by influenza A), each of which required a course of etoposide. [b] Weeks on etoposide are counted from the first etoposide dose to 1 week after the last dose. [c] No infectious agent identified. [d] *Streptococcus mitis* in blood culture. [e] 95% autologous reconstitution. CNS: central nervous system; CSA: cyclosporine A; IVIG: intravenous immunoglobulin; HCQ: hydroxychloroquine; HLH: hemophagocytic lymphohistiocytosis; HSCT: hematopoietic stem cell transplantation; ICU: intensive care unit; MP: methylprednisolone; MAS: macrophage activating syndrome; PCP: *Pneumocystis jiroveci* (previously known as *carinii*) pneumonia; TCZ: tocilizumab.

infected with EBV and developed fulminant HLH (ferritin 121,937 µg/L, EBV-DNA 1.26 × 10^6 copies/mL) with severe CNS involvement (disorientation, seizures, highly pathological EEG with findings of a generalized encephalopathy, and an abnormal MRI). Despite MP pulses, her CNS affection rapidly deteriorated and her HLH was severe. Consequently, therapy was intensified with etoposide (initially 100 mg/m^2 once weekly and later 150 mg/m^2 once weekly) and in addition, RTX. Her neurological symptoms regressed within 5 days. She recovered fully and a subsequent MRI was normal.

· *Patient 3.* A 3-year-old girl with sJIA was on treatment with TCZ, subcutaneous MTX, and oral prednisolone, but following a varicella-zoster virus infection, the immunosuppressive therapy was stopped. Subsequently, she flared in her sJIA and developed MAS-HLH. Despite MP pulses, the patient showed progressive neurological symptoms including seizures and finally unconsciousness. EEG showed encephalitis and an MRI revealed widespread findings in accordance with MAS-HLH. Due to the combination of progressive HLH and very severe neurological findings with abnormal MRI and EEG, etoposide therapy (100 mg/m^2) was added. Within 4 weeks of etoposide therapy the girl was awake and could communicate, but still had visual and motor impairments. Notably, she recovered fully.

· *Patient 5.* A 15-year-old girl with SLE rapidly developed MAS-HLH with neutropenia (< 0.1 × 10^9/L) and CNS involvement (convulsions, choreoathetotic movements, and reduced consciousness). EEG showed generalized slow activity and MRI revealed changes in accordance with MAS-HLH. Despite MP pulses, she had a progressive brain edema with intracranial pressure of 40 mmHg and Glasgow Coma Scale 3. Due to very severe CNS involvement, etoposide was added, initially at a markedly reduced dose (50 mg/m^2) due to neutropenia, but subsequently increased to 75–100 mg/m^2. Within 2 weeks of etoposide, she was awake with open eyes, spontaneous motor movements, but no cognitive contact. She improved further but had multiple infarctions on MRI and developed severe neurological sequelae.

· *Patient 6.* A 5-month-old girl was diagnosed with atypical sJIA and initially treated with anakinra, after treatment with prednisolone and MP pulses failed. She suffered recurring MAS-HLH episodes; the first episode when etoposide was given

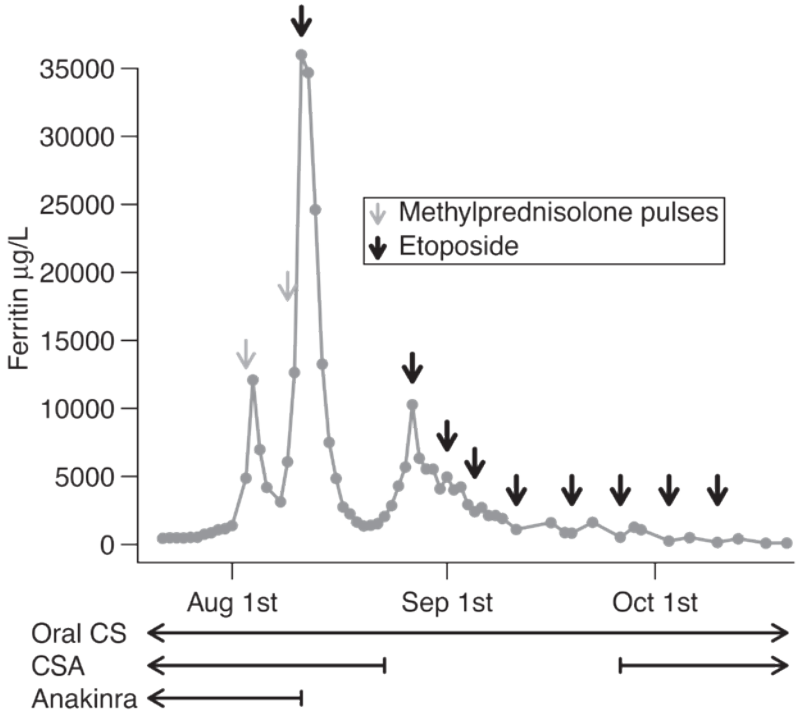

Figure 1. Prompt reduction of ferritin levels after administration of etoposide in a patient with MAS-HLH. Despite treatment with oral CS, CSA, anakinra, and multiple high-dose MP pulses (3 + 2 days) in this 5-year-old boy (Patient 4) with systemic juvenile idiopathic arthritis, ferritin levels were rapidly increasing (max value 36,007 µg/L), while blood counts were dropping (neutrophil count 0.3×10^9/L prior to etoposide start). Within 24 hours of the first etoposide dose, the dramatic increase of ferritin was halted, and 2 days later the ferritin level was reduced to one-third. Altogether, 9 doses of etoposide were administered: 7 doses at 100 mg/m^2 and 2 doses at 50 mg/m^2 (doses 2 and 3). The boy had had 1 previous MAS flare, with a peak ferritin level of 15,678 µg/L (May 3), treated with MP pulses in addition to oral CS, anakinra, and CSA; the latter 3 treatments were continued thereafter. Anakinra was stopped the day of the first etoposide dose, and CSA 10 days later. CS: corticosteroids; CSA: cyclosporine A; MP: methylprednisolone.

was due to insufficient response to corticosteroids and CSA. The second episode was the most severe with ferritin > 40,000 µg/L, IL-18 210,000 ng/L, and concurrent influenza A. She deteriorated with dyspnea, lethargy, high fever, tachycardia, generalized edema, and hypertension. Pericardial effusion and PAH were noted. Since she did not respond to MP pulses, etoposide was again initiated with prompt response. The third episode was milder, and only 2 doses were administered. A genetic etiology could not be identified by trio whole genome sequencing. Due to recurring MAS-HLH, she underwent an allogeneic HSCT with 95% autologous reconstitution but without disease reactivation.

· *Patient 7.* A 16-year-old girl with atypical sJIA on treatment with anakinra developed MAS-HLH together with pulmonary symptoms. After 3 days of MP pulses, her breathing difficulties progressed, she produced voluminous yellow secretions from the airways, CT scan showed diffuse lung infiltrations and pleural effusions, and she required mechanical ventilation. Ferritin (26,000 µ/L) and IL-18 (230,000 ng/L) levels increased. Since she deteriorated despite MP pulses, etoposide 50 mg/m^2 was initiated with prompt clinical and laboratory improvement. One week later she developed posterior reversible encephalopathy syndrome (PRES), successfully treated with antihypertensive and antiepileptic drugs. Etoposide was paused but after 2 weeks, her sJIA flared with persisting MAS-HLH and another episode of PRES with seizures despite MP pulses and IV immunoglobulin; this prompted continued treatment with etoposide. She gradually improved and her MAS-HLH was gone 5 weeks after initiating etoposide treatment. The girl recovered fully without any neurological late effects.

DISCUSSION

In this report, addition of moderately dosed etoposide to treat severe MAS-HLH refractory to conventional antiinflammatory therapy achieved fast control of the cytokine storm. The regimen was generally well tolerated.

In this case series, we utilized our experience in primary and secondary HLH.[3,4,11] Etoposide has a unique ability to suppress HLH-like immunopathology and inflammatory cytokine production by potently ablating activated T cells.[12] Case reports on etoposide for MAS-HLH have shown rapid recovery without serious adverse events.[13]

CNS involvement is frequent in HLH, and may lead to

irreversible neurological damage.[3,4] Five children had CNS involvement at initiation of etoposide treatment, but despite being very severe in 3, only 1 child suffered long-term neurological sequelae, suggesting that etoposide can also effectively reduce CNS affection in MAS-HLH. Reports on fatal severe pulmonary disease in MAS-HLH have also emerged.[14] A recent study of 61 patients with sJIA and parenchymal lung disease revealed a 5-year survival of 58% and the predominant pathology of PAP (as in Patient 4) and/or endogenous lipoid pneumonia.[15]. Our 5 patients with pulmonary involvement all survived, suggesting a possible positive effect of etoposide on the pulmonary disease. Our positive experience of etoposide in MAS-HLH is in line with a report on 89 adult patients with MAS-HLH for whom etoposide and cyclophosphamide were reported to have the best efficacy.[16]

There is a risk of secondary malignancies, especially acute myeloid leukemia, associated with etoposide, in particular with cumulative etoposide doses[17] > 2000 mg/m^2. However, according to the International Agency for Research on Cancer, this risk of carcinogenicity is mainly when etoposide is given in combination with cisplatin and bleomycin; there is limited evidence for carcinogenity of etoposide alone.[18] Our patients received a limited median cumulative dose of etoposide of 800 mg/m^2 (range 300–1050 mg/m^2). We therefore conclude that the risk of secondary malignancies following moderately dosed etoposide is very limited and not a contraindication for its use in MAS-HLH. On the contrary, we deem that with a mortality risk of 5–10%, the benefits of etoposide well outweigh the risks in treatment of severe MAS-HLH.

The exact pathogenesis of MAS-HLH remains to be elucidated. It is known that infections may trigger the development of MAS-HLH[19] and that the immune system's inability to downregulate an initially physiological inflammatory response to a trigger creates an escalating state of excessive inflammation (hyperinflammation). The immune dysregulation of macrophages and lymphocytes leads to a cytokine storm.[20] An imbalance in IL-18/IL-18 binding protein levels, with increased systemic levels of free bioactive IL-18, is of importance for the development of MAS-HLH.[21]

The abnormalities in lymphocyte cytotoxicity in MAS-HLH have no obvious explanation but an inflammation-induced NK cell exhaustion, mediated through the constitutively high levels of cytokines (IL-18 and IL-6), may contribute to MAS-HLH by diminishing NK cell-mediated immunoregulation of inflammatory responses.[22] Interestingly, reduced NK cell numbers and cytotoxicity has also been reported in pediatric sepsis,[23] and in a set of critically ill adult patients, increased ferritin levels (i.e., hyperinflammation) were associated with decreased lymphocyte cytotoxicity and degranulation.[24] Moreover, as reviewed by Schulert and Canna, a combination of quantitative defects in NK cell numbers and qualitative defects in perforin expression have been reported in MAS-HLH.[19] Grom, *et al* described immunologic abnormalities in cytotoxicity in 7 patients with sJIA who had developed MAS-HLH.[25] In our 7 patients with MAS-HLH, we found low percentages of NK cells in 5 of the 7 children analyzed prior to or in association with diagnosis, and associated low NK cell activity in 2 of 5 children studied (Supplementary Table 1, available with the online version of this article).

The use of IL-1 inhibition in high dosage for MAS-HLH is expanding and a randomized, double-blinded, placebo-controlled trial is ongoing (ClinicalTrials.gov: NCT02780583). IL-6 inhibition may also be valuable. In addition, MAS-HLH trials are currently ongoing with interferon-γ inhibition (NCT03311854) and IL-18 inhibition (NCT02398435). Targeted inhibition of Janus kinase signaling may also be beneficial (NCT04120090).

Our report has limitations. First, there may be patients with MAS-HLH during this period treated with non-etoposide–based regimens. Second, we report few patients, treatment was not randomized, and data were collected retrospectively. Nevertheless, the effect of adding etoposide to conventional treatment was remarkably positive.

To conclude, while conventional treatments and anakinra are effective in most cases of MAS-HLH,[9] moderately dosed etoposide remains a relevant choice in (1) severe rapidly progressive, fulminant, or refractory MAS-HLH despite anakinra treatment, and (2) MAS-HLH with CNS involvement when an immediate effect is essential, and then possibly in combination with anakinra. Etoposide is very affordable and easily accessible. Moreover, the dose and dosing frequency can easily be individualized, and stopped when the hyperinflammation and CNS involvement are sufficiently reduced; treatment can probably be stopped earlier than we did in most of our patients. We suggest an optimized, stratified treatment approach of MAS-HLH, and the establishment of an international MAS-HLH registry.

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ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this article.

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