Efficacy of moderately dosed etoposide in macrophage activation syndrome - hemophagocytic lymphohistiocytosis (MAS-HLH)

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Short title: Etoposide in MAS-HLH

Word count: 2 500. Abstract: 100. References: 25. Tables: 2. Figures: 1.
Supplementary Text: 1. Supplementary Table: 1.

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Key words: Juvenile idiopathic arthritis, systemic lupus erythematosus, macrophage activation syndrome, hemophagocytic lymphohistiocytosis, etoposide

The sources of support in the form of grants or industrial support:

The study was supported by grants from the Swedish Children's Cancer Foundation, the Swedish Cancer Foundation, the Swedish Research Council, Cancer and Allergy Foundation of Sweden, and Region Stockholm (ALF-project) to J.I.H. and Swedish Children's Cancer Foundation, the Swedish Cancer Foundation, the Swedish Research Council, and the Swedish Foundation for Strategic Research to Y.T.B. The funding bodies had no role in the design of the study and collection, analysis, and interpretation of data or in writing the manuscript.

Conflict of interest:

The authors have no conflicts of interest to declare.

ABSTRACT

Objective: Macrophage activation syndrome (MAS) constitutes one 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 to 5-10%. Here we report our experiences with moderately dosed etoposide in severe MAS-HLH, administered with the objective to effectively reduce severe hyperinflammatory activity with limited side effects.

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

Results: All children promptly responded to moderately dosed etoposide (50-100 mg/m^2 once weekly), added to conventional MAS-HLH treatment which was considered insufficient. The mean accumulated etoposide dose was 671 mg/m^2 (range 300-1,050 mg/m^2), as compared to 1,500 mg/m^2 recommended the first 8 weeks of the HLH-94/HLH-2004 protocols. One child developed neutropenic fever and another neutropenic sepsis (neutrophils 0.3x10^9/L at therapy onset). Five/seven children had low percentages (<5%) circulating NK-cells prior to or in association with diagnosis; NK-cell activity was pathologically low in two/five children studied. Disease-causing variants in HLH-associated genes were not found. All children were alive at latest follow-up (2-9 years after onset); neurological symptoms had normalized in four/five affected children.

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

INTRODUCTION

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

MAS constitutes one subtype of the hyperinflammatory syndrome hemophagocytic lymphohistiocytosis (HLH), characterized by fever, hyperferritinemia, cytopenia, and coagulopathy, which may progress to multi-organ failure(2). HLH includes primary (familial/genetic) and secondary (acquired) HLH (sHLH)(2). HLH in patients with underlying autoimmune or 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 around 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, while 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(5-7).

For MAS-HLH most treating physicians use high-dose corticosteroids and CsA(1). Furthermore, treatment with the IL-1 inhibitor anakinra is rapidly expanding(8), and many patients respond to 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 seven children with MAS-HLH for whom rapid deterioration, CNS and/or pulmonary affection prompted us to administer etoposide in addition to conventional anti-inflammatory 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. Moreover, genetic and lymphocyte cytotoxicity analyses were performed.

PATIENTS AND METHODS

We report seven children with MAS-HLH (three sJIA, two atypical sJIA, and two SLE), aged 0.4–16 years (median 9) at MAS-HLH onset, treated with etoposide during 2010-2017; four at Karolinska University Hospital, Stockholm, two at Sahlgrenska University Hospital, Gothenburg, and one at Umeå University Hospital, the latter three in collaboration with Karolinska University Hospital. Clinical and laboratory data was 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 labelled as "atypical", as they did not have arthritis at diagnosis. Both SLE patients fulfilled the 1997 American College of Rheumatology revised SLE criteria. All fulfilled the diagnostic guidelines for MAS for children with sJIA and SLE, respectively; four also fulfilled the HLH-2004 diagnostic criteria(3).

The Ethics Committee, 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 Text.

RESULTS

Patient demographics, clinical characteristics and laboratory findings are summarized in Table 1. For additional patient information, see below.

Severity of MAS-HLH

All children were severely ill and had CNS involvement or did not respond to conventional MAS-HLH therapy, whereof six 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, MRI showing CNS inflammation, and EEG indicating encephalitis. Patient 7 had two 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 three 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 was then diagnosed with pulmonary arterial hypertension. Patient 7 displayed voluminous yellow airway secretions; CT scan showed diffuse lung-infiltration and pleural effusions.

MAS-HLH treatments

All initially received intravenous pulse methylprednisolone followed by oral steroids. Additionally, CsA was given to two and anakinra to three (up to 2.7-15 mg/kg/day) (Table 2). Nevertheless, severe MAS-HLH activity remained and, therefore, intravenous etoposide was initiated. For clinical parameters 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 EBV infection, received 150 mg/m^2 once weekly from week 4 and, additionally, rituximab (Table 2). Patient 6 had three separate etoposide-treated MAS-HLH episodes. The total number etoposide doses administered varied between four and eleven. 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 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 x10^9/L at start). Patient 2 developed neutropenic fever.

Lymphocyte cytotoxicity and genetic analyses

Five of seven children had low percentages (<5%) circulating NK-cells in PBMC prior to or in association with diagnosis, of which three also had low absolute NK-cells numbers (<0.07x10^9/L). NK-cell activity was pathologically low (≤10 lytic units) and NK-cell degranulation defective (<5%) in two children with NK lymphopenia (Supplementary Text; Supplementary Table).

Genetic analyses were performed in six patients. Selected HLH-associated genes were analyzed in four patients, whereas whole genome sequencing was performed in two patients. No suspected pathogenic variants were identified in HLH-associated genes in any of the patients (Supplementary Text; Supplementary Table).

Patient descriptions

The clinical course and treatment in patients 1-3 and 5-7 is 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 16-year previously healthy boy with a 4-month history of SLE developed accelerating inflammatory disease (ferritin 20,778 µ/L), but whilst his laboratory values improved markedly following methylprednisolone (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, he recovered fully and a subsequent MRI was normal.

Patient 2: A 9-year old girl with sJIA on treatment with tocilizumab and oral methotrexate was infected with EBV and developed fulminant HLH (ferritin 121,937 µg/L, EBV-DNA 1.26x10^6 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 rituximab. 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 tocilizumab, subcutaneous methotrexate and oral prednisolone, but following a VZV-infection the immunosuppressive therapy was stopped. Subsequently, she flared in her sJIA and developed MAS-HLH. Despite MP pulses the girl 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, 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 (<0.1x10^9/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 the 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, but after treatment failure with tocilizumab and MP-pulses. She suffered recurring MAS-HLH episodes, the first treated with etoposide due to insufficient response to corticosteroids and cyclosporine A. 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 pulmonary arterial hypertension were noted. Since she did not respond to MP pulses, etoposide was again initiated with prompt response. The third episode was milder, and only two doses were administered. A genetic etiology could not be identified by Trio WGS. 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 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 anti-epileptic 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 IVIG, prompting 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 anti-inflammatory 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 three, only one 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 being PAP (as in patient 4) and/or endogenous lipoid pneumonia(15). Our five 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 MAS-HLH patients, for whom etoposide and cyclophosphamide were reported to have best efficacy(16).

There is a risk of secondary malignancies, especially acute myeloid leukemia, associated with etoposide, in particular with cumulative etoposide doses exceeding 2,000 mg/m^2(17). However, according to International Agency for Research on Cancer, this risk of carcinogenicity is mainly when etoposide is given in combination with cisplatin and bleomycin while 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 300-1,050 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-18BP 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 via 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 & Canna, a combination of quantitative defects in NK-cell numbers and qualitative defects in perforin expression have been reported in MAS-HLH(3). Grom *et al.* described immunologic abnormalities in cytotoxicity in seven sJIA patients that had developed MAS-HLH(25). In our seven MAS-HLH patients, we found low percentages of NK-cells in five of the seven children

analyzed prior to or in association with diagnosis, and associated low NK-cell activity in two of five children studied (Supplementary Table).

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 IFN-gamma inhibition (NCT03311854) and IL-18 inhibition (NCT02398435). Targeted inhibition of Janus kinase (JAK)-signaling may also be beneficial (NCT04120090).

Our report has limitations. Firstly, there may be MAS-HLH patients during this period treated with non-etoposide based regimens. Secondly, we report few patients, treatment was not randomized, and data was 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 (a) severe rapidly progressive, fulminant or refractory MAS-HLH despite anakinra treatment, and (b) in MAS-HLH with CNS involvement when prompt effect is essential; 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, and earlier than we did in most of our patients. We suggest an optimized, stratified treatment approach of MAS-HLH, and to establish an international MAS-HLH registry.

ACKNOWLEDGEMENTS

The authors are grateful to the patients and their parents for participating in this study. JIH initiated the treatment with moderately-dosed etoposide in this patient series. ACH and JIH initiated and designed the study, included patients, and reviewed and structured patient data. TvBG and UA contributed to study conception and design. CB, SB, ME, PE, and SH included patients. SCCC performed cytotoxicity analyses, supervised by YTB who also interpreted data. MM interpreted genetic data. ACH drafted the article, together with TvBG and JIH, and all authors critically revised it, and read and approved the final version.

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FIGURE LEGEND

Figure 1: Prompt reduction of ferritin levels after administration of etoposide in a patient with MAS-HLH. Despite treatment with oral corticosteroids (CS), cyclosporine A (CsA), anakinra and multiple high-dose methylprednisolone (MP) pulses (three plus two 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 x10^9/L prior to etoposide start). Within 24 hours of the first etoposide dose the dramatic increase of ferritin was halted, and two days later the ferritin level was reduced to one third. Altogether nine doses of etoposide were administered; seven doses at 100 mg/m^2 and two doses at 50 mg/m^2 (doses 2 and 3). The boy had had one previous MAS-flare, with a peak ferritin of 15,678 µg/L (May 3rd), treated with MP-pulses in addition to oral CS, anakinra and CsA, the latter three treatments continued thereafter. Anakinra was stopped the day of the first etoposide dose, and CsA ten days later.

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

Patient number	1	2	3	4	5	6 *	7
Sex	Male	Female	Female	Male	Female	Female	Female
Parental consanguinity	No	No	No	No	No	No	No
Age at onset of MAS-HLH	16 yr	9 yr	3 yr	5 yr	15 yr	5 mo	16 yr
Underlying disease	SLE	sJIA	sJIA	sJIA	SLE	Atypical sJIA	Atypical sJIA
Time disease onset to MAS-HLH	6 mo	5 yr	10 mo	3 mo	1 mo	5 mo	7 mo
Previous treatment	Oral steroids, hydroxychloroquine	Oral steroids, MP-pulses, etanercept	Etanercept, MP-pulses	Oral steroids, IVIG, CsA, anakinra, MP-pulses	Oral steroids, hydroxychloroquine	Anakinra, tocilizumab, MP-pulses	Oral steroids, anakinra
Ongoing treatment at the time of MAS-HLH diagnosis	Oral steroids, hydroxychloroquine	Tocilizumab, MTX	Oral steroids, tocilizumab, MTX	Oral steroids, CsA, anakinra	Oral steroids, hydroxychloroquine	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 (Ravelli)	N/A	Yes	Yes	Yes	N/A	Yes	Yes
MAS criteria SLE (Parodi)	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 x 10^9/L)	3.3	6.4	3.2	0.3	0.4	11.8	7.9
- Platelets (< 100 x 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)	2 984	121 937	25 946	36 007	12 558	5 025	15 000
- sCD25 levels (> 2 400 U/ml)	2 172	>7 500	>7 500	3 309	3 460	ND	ND
- NK-cell activity defective**	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

* Patient 6 had in total three episodes with MAS (one triggered by influenza A) which each resulted in a course of etoposide. ** In first sample analyzed; NK-cell activity defective <10 lytic units.
yr=years; mo=months; N/A=not applicable; MAS-HLH=Macrophage Activating Syndrome-Hemophagocytic lymphohistiocytosis,; SLE=Systemic lupus erythematosus; sJIA=Systemic Juvenile Idiopathic Arthritis, Pred=Prednisone; MP=Methylprednisolone; IVIG=intravenous immunoglobulins; CsA=Cyclosporine A; MTX=methotrexate(subcutaneous); EBV=Epstein-Barr virus; VZV=Varicella zoster virus; UTI=Urinary tract infection; sCD25= Soluble interleukin-2 receptor; NK=Natural killer; CNS=central nervous system; MRI= Magnetic resonance imaging; EEG= electroencephalogram.

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

Patient number	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		Hypertonia	Kidney stones, hydronephrosis	No	No	No
Etoposide	75 mg/m^2 x 4	100 mg/m^2 x 3 150 mg/m^2 x 5	100 mg/m^2 x 9	50 mg/m^2 x 2 100 mg/m^2 x 7	50 mg/m^2 x 3 75 mg/m^2 x 2 100 mg/m^2 x 2	50-75 mg/m^2 x 4* 75 mg/m^2 x 5* 75 mg/m^2 x 2*	50 mg/m^2 x7
Weeks on etoposide§	4	8.5	8	9.5	6	4+6+2*	8
Accumulated etoposide dose (mg/m^2)	300	1050	900	800	500	800	350
Toxicities during etoposide therapy:							
Neutrophils <0.5 x10^9 (days)	0	4	2	18	6	0	0
Platelets <50 x109 (days)	0	8	0	0	2	0	0
Platelets <20 x10^9 (days)	0	1	0	0	0	0	0
Neutropenic fever and/or severe infections	0	Neutropenic fever[a]	0	Neutropenic sepsis[b]	0	0	0
Infection prohylaxis	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	Hydroxychloroquine	None	Anakinra, Tocilizumab	6-mercaptopurine, CSA	Azathioprine, hydroxychloroquine	None	Tocilizumab, CsA
HSCT	No	No	No	No	No	Allogeneic HSCT	No
Outcome and Follow-up	Alive 9 yr after onset	Alive 6 yr after onset	Alive 8 yr after onset	Alive 8 yr after onset	Alive 6 yr after onset	Alive 2 yr after HSCT	Alive 2 yr after onset
Treatment at last follow up	Hydroxychloroquine	None	None	None	Hydroxychloroquine	None	Tocilizumab
Clinical response	Complete	Complete	Complete	Complete	Severe CNS sequelae	Complete#	Complete

* Patient 6 had in total three episodes with MAS (one triggered by influenza A) which each resulted in a course of etoposide; # 95% autologous reconstitution; § Weeks on etoposide are counted from the first etoposide dose to one week after the last dose; a) no infectious agent identified; b) streptococcus mitis in blood culture. MP= Methylprednisolone; TMP/SMX=trimethoprim/sulfamethoxazole; CsA= Cyclosporine A; IVIG=intravenous immunoglobulins; HSCT=hematopoietic stem cell transplantation