The influence of various therapeutic regimens on early clinical and laboratory response and outcome of children with secondary hemophagocytic lymphohistiocytosis

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Abstract

Introduction: Secondary hemophagocytic lymphohistiocytosis (sHLH) is a life-threatening syndrome of severe hyperinflammation which is often triggered by infection or autoimmune disease (macrophage activation syndrome – MAS). The aim of our study was to assess the frequency of sHLH/MAS in children treated in our institution and to compare the effectiveness of various therapeutic interventions.

Material and methods: Between 2005 and 2013, 24 children (age: 1–17 years) were consecutively treated for sHLH/MAS. Therapy was based on glucocorticoids (GCs) in high or standard doses (hd-GCs or sd-GCs), intravenous immunoglobulin (IVIG), and cyclosporin A (CyA). A comparison of selected laboratory and clinical parameters during the first 72 h of treatment and after a week from the last intervention applied in the first 72 h after diagnosis was performed retrospectively.

Results: The majority of patients (14/24, 58%) suffered from sHLH/MAS in the course of an autoimmune disease (12 patients diagnosed with a systemic form of juvenile idiopathic arthritis). We found with a confidence level of 95% that the application of hd-GCs in the first 24 h caused rapid alleviation of fever, reduction of hepatosplenomegaly, and an increase in thrombocytes and s-fibrinogen concentrations. The use of combination therapy with hd-GCs, IVIG, and CyA in the first 72 h caused a faster increase in s-fibrinogen. All patients survived and were alive at the follow-up of 1–8 years.

Conclusions: The results indicate that treatment of sHLH/MAS based on hd-GCs, CyA and IVIG is an effective therapy in children.

Key words: hemophagocytic lymphohistiocytosis, macrophage activation syndrome, juvenile idiopathic arthritis, children.

Introduction

Hyperinflammatory syndromes caused by massive cytokine release from activated immune cells in response to various congenital and acquired factors encompass an infrequent, yet potentially life-threatening group of disorders [1, 2]. Macrophages and cytotoxic CD8+ lymphocytes massively release various inflammatory mediators (e.g., INF-γ, TNF-α,

IL-1, IL-6), and hematopoietic growth factors [3, 4]. Aforementioned phenomena together with impaired cytotoxic function of natural killer (NK) cells and CD8+ lymphocytes results in the hyperinflammatory phenotype [1].

The clinical syndrome of the exaggerated inflammatory reaction is called hemophagocytic lymphohistiocytosis (HLH) or hemophagocytic syndrome [1]. Depending on the etiology, HLH can be divided into genetic and acquired (secondary HLH – sHLH) forms [1, 2, 5]. Inherited forms of HLH usually present in infancy or early childhood (80% of cases). Secondary HLH develops at any age as a consequence of immune activation caused by infection, autoimmune disorder or malignancy (I-HLH, A-HLH or M-HLH) [5–7].

The hyperinflammatory response may be the cause of long-lasting unexplained fever [1]. Besides fever, the most common symptoms of HLH are splenomegaly and peripheral blood cytopenia [1, 5]. Hepatomegaly, lymphadenopathy, neurological symptoms, edema or skin rash may also be present. Biochemical hallmarks of HLH include hyperferritinemia, increased level of the α chain of the soluble receptor for interleukin-2 (sIL-2Rα; sCD25), hypertriglyceridemia, hypofibrinogenemia, coagulopathy, hyponatremia, and elevated liver transaminases and bilirubin [3]. High levels of sIL-2Rα in serum are seen in all forms of HLH [5]. Although sIL-2Rα and hyperferritinemia are markers of generalized inflammation, ferritin levels > 3000 µg/l raise suspicion for HLH, and levels > 10,000 µg/l are highly suspicious [8]. Cytohistological examination of the affected tissues may reveal hemophagocytosis [1].

Mechanisms of acquired HLH are not completely understood. Some patients have at least transient defects in NK cell function or have a single copy of a known gene mutation in HLH [9, 10]. Recent studies in animal models suggest enhanced antigen presentation and excessive signaling of Toll-like receptors as mechanisms of sHLH [11].

Autoimmune-associated HLH, often referred to by many rheumatologists as macrophage activation syndrome (MAS), has most often been reported in the systemic form of juvenile idiopathic arthritis (7–10% of patients) but can also occur in the course of other autoimmune diseases [11–13]. A-HLH/MAS may present as a life-threatening disease; symptoms are often difficult to distinguish from an exacerbation of the patient's underlying autoimmune disease or triggering infection, which increases the risk of delaying diagnosis and appropriate therapy [12–15].

So far, treatment of A-HLH/MAS is not standardized and remains highly variable across clinical centers [16]. A frontline treatment of MAS, particularly of milder grades, involves glucocorticoids with or without intravenous immunoglobulin (IVIG), which may be sufficient to control hyperinflammation [13]. Immunochemotherapy (including etoposide) based on the protocol HLH-2004 has an associated 5-year mortality of 54% of patients, and it is believed that it may favorably affect the outcome in severe cases of sHLH/MAS [1].

The aim of our study was to assess the frequency of sHLH/MAS in children treated in our institution for non-malignant sHLH and to compare the effectiveness of various therapeutic interventions.

Material and methods

The study population includes 24 children aged 1–17 years, who were consecutively treated for non-malignant secondary HLH at the Department of Pediatrics, the Children's Memorial Health Institute in Warsaw, between January 2005 and December 2013. All patients were referred with the following indications: generalized inflammatory reaction of unknown etiology (SIRS)/sepsis/fever of unknown origin (FUO), and suspected systemic connective tissue disease.

The diagnosis of HLH was based on the criteria proposed by the HLH Study Group of the Histiocyte Society in 2004 (HLH-2004) and in some cases on Ravelli's criteria [5, 17]. According to the HLH-2004, at least 5 of 8 diagnostic criteria must be fulfilled for HLH diagnosis: (1) fever, (2) splenomegaly, (3) cytopenia affecting $\geq$ 2 cell lines (hemoglobin < 90 g/l, and in infants < 100 g/l; platelet count < 100×10^9/l; neutrophils < 1.0×10^9/l), (4) hyperferritinemia (> 500 µg/l), (5) hypertriglyceridemia (fasting triglycerides > 3.0 mmol/l) and/or hypofibrinogenemia (< 1.5 g/l), (6) hemophagocytosis in bone marrow, spleen, liver, or lymph nodes, (7) elevated level of sIL-2Rα > 2400 U/ml, (8) low/absent NK cell activity [5]. Exclusion of primary HLH was performed using functional tests and molecular genetics modalities according to standard practice [1].

The diagnosis of systemic connective tissue diseases was determined at the time of the diagnosis of HLH or during follow-up in the Rheumatology Institute in Warsaw.

Therapies used for sHLH have been evaluated. A frontline therapy of HLH has included high-dose glucocorticoids (GCs-hd) or standard-dose glucocorticoids (GCs-sd) with methylprednisolone (10–30 mg/kg/day or 1–2 mg/kg/day, respectively), with or without addition of IVIG (1–2 g/kg/day) and/or cyclosporine A (CyA) (3–5 mg/kg/day). Additionally, patients with severe HLH were treated with etoposide (100–150 mg/m^2/dose *i.v.*) or biological response modifiers (infliximab). Some of them required therapy in the intensive care unit (ICU) and hemodiafiltration. The treatment was individualized, depending on the patient's clinical

condition (if the clinical status of the child was more serious then the therapy was started faster) or currently used routines in the department.

The influence of particular HLH therapy on clinical symptoms – fever, splenomegaly and hepatomegaly (assessed by computed tomography or ultrasound), and laboratory markers of HLH (s-ferritin, s-fibrinogen, and whole blood thrombocytes) – was retrospectively studied.

Response to the therapy was analyzed depending on type of the used treatment and the time point for its introduction in relation to the diagnosis of HLH. According to the above, the patients were divided into the following subgroups:

a) that had received GCS-hd within the first 24 h,
b) that had not received GCS-hd within the first 24 h,
c) that had received GCS-hd within the first 72 h,
d) that had not received GCS-hd within the first 72 h,
e) that had received GCS-hd together with IVIG, CsA,
f) that had received GCS-hd together with CsA,
g) where other treatments, but different to GCS-hd, were applied.

Two time points were established, with respect to which the behavior of the measured parameters was monitored. T1 was the third day of the combined action of the interventions applied in the first days from diagnosis. T2 was the seventh day after the last intervention applied during the first 72 h after diagnosis. If within 7 days of administration of the last intervention in the first 72 h, other interventions were used, T2 was taken as the point after 72 h of administration of the last intervention during the first 7 days after diagnosis.

Statistical analysis

The laboratory parameters examined have numeric values – ferritin and fibrinogen concentration and platelet count; clinical parameters were presented as discrete numbers of days after which a given symptom abated after the application of a given intervention (i.e., resolution of fever, hepatomegaly, and splenomegaly).

Percentage values are shown for patients in given groups, range, average, standard deviation for the variable describing the percentages (in the context of the magnitude of changes) by which there was a decrease in the concentration of ferritin, an increase in the concentration of fibrinogen, a decline in the number of platelets, or an abatement of fever, hepatomegaly and splenomegaly – these were accepted as endpoints.

It was then evaluated whether the percentage of patients (named as p1) in whom the endpoint had been reached in the period T1–T2 in one of the groups a-f was significantly higher than the percentage of patients (named as p2) in the other groups ((a) vs. (b); (c) vs. (d); (e) vs. (g)). The verification whether p1 was significantly higher than p2 was made on the basis of the methods implemented and available in the package ExactCldiff, environment R, depending on the identification of the confidence intervals at the confidence level $(1 - \alpha)$ for the difference of percentages p1 – p2. Statistical methods were based on construction of the smallest one-sided confidence interval for the difference between two proportions [18, 19].

A positive difference ($p1 - p2 > 0$) led to the conclusion that at a significant α level, it can be concluded that p1 was significantly higher than p2. The number of days for fever to abate was considered as a quantitative variable.

On the basis of the Wilcoxon test, it was assessed whether there was a difference in time for the abatement of fever for each pair: (a) vs. (b), (c) vs. (d), (e) vs. (g).

The study protocol was developed according to the ethical standards of the Declaration of Helsinki and approved by the institutional ethics committee at the Children's Memorial Health Institute. All patients or legal guardians provided their written informed consent.

Results

In all 24 studied patients (17 girls and 7 boys), HLH was associated with autoimmune disease, infection or unidentified trigger. The majority of patients (14/24, 58%) suffered from HLH in the course of an autoimmune disease: 12 patients were diagnosed with a systemic form of juvenile idiopathic arthritis (sJIA), 1 patient had systemic lupus erythematosus, and another 1 had Kawasaki disease. In almost all of them, MAS was the first manifestation of systemic connective tissue diseases (13/14, 93%). Six patients suffered from a one-time episode of HLH of unknown etiology, and in 4 patients HLH was identified in the course of infection without diagnosis of systemic disease in the annual follow-up. The detailed causes of sHLH in the analyzed group and clinical and biological characteristics are presented in Tables I and II.

The most common therapeutic interventions used were pulses of methylprednisolone (n = 23, 96% of patients), CyA (n = 21, 88%), and IVIG (n = 19, 79%). Standard glucocorticoid doses were used in 29% of patients. Over one third of patients required supportive treatment in the ICU (n = 9, 38%). Rarely (n = 2, 8% of patients) other interventions were applied (etoposide, infliximab, hemodiafiltration) (Figure 1).

All patients were treated with empirical broad spectrum antibiotics. In the first 72 h after the diagnosis of HLH, at the same time, or with a small interval, within 72 h, the most common interven-

Table I. Causes of sHLH/MAS and triggering factors

Primary disease-inducing agents	Number of patients (%)
MAS (systemic juvenile idiopathic arthritis)	12 (50)
MAS (Kawasaki disease)	1 (4)
MAS (systemic lupus erythematosus)	1 (4)
sHLH/MAS as the first manifestation of autoimmune disease	13/14 (93)
Infection-associated HLH:	4 (17)
Mycobacterium	1 (4)
Borrelia	1 (4)
Parvovirus B19	1 (4)
EBV	1 (4)
HLH of unknown etiology	6 (25)
Possible infectious trigger of sHLH/MAS:	17 (71)
EBV	4 (16)
CMV	2 (8)
Others (*Mycoplasma*, *Yersinia*, *Toxocara*, *Staphylococcus* spp., *Streptococcus* spp., *Rotavirus*)	9 (38)
Etanercept as possible sHLH/MAS trigger	1 (4)
Unidentified sHLH/MAS triggers	8 (33)

sHLH/MAS – secondary hemophagocytic lymphohistiocytosis/macrophage activation syndrome, GCs-hd – high-dose glucocorticoids, GCs-sd – standard-dose glucocorticoids, CyA – cyclosporine A, IVIG – intravenous immunoglobulins, ICU – intensive care unit, EBV – Epstein-Barr virus, CMV – cytomegalovirus.

tions were associated with treatment with pulses of methylprednisolone, IVIG and CyA (n = 7, 29%) and pulses of GCs together with CyA (n = 5, 20% of patients). Twelve (50%) patients received a pulse of GCs in the first 24 h after diagnosis, 17 (71%) during the first 72 h. Seven patients (29%) did not receive a pulse of GCs in the first 72 h.

Patients required therapy in the ICU on average after 72 h from the diagnosis of HLH, the earliest within the first 48 h, 50% on the second or third day after diagnosis of HLH, the remainder 50% later, but not later than on the seventh day after diagnosis.

All of the patients (n = 24, 100%) were alive, during the early and late follow-up (1 to 8 years). There were no infectious complications when steroid therapy was used, long-term complications, or recurrence of HLH; 14 patients remain under rheumatologic care, 12 of them diagnosed with sJIA; in 8 patients transient symptoms of iatrogenic Cushing's syndrome were observed.

Time point T1 most often was found on the third or fourth day after diagnosis, point T2 on the seventh to ninth day. A detailed presentation of time points for each patient, the type of intervention, and laboratory and clinical parameters values are presented in Tables III and IV. In Table V we present the percentages of patients and basic statistical data for the variables describing the endpoints and in Tables VI and VII the confidence intervals for the difference of patients with improvement of the parameter for each of the three pairs of groups.

It was found, with a confidence level of 90%, that the percentage of patients with an increase in the number of thrombocytes and fibrinogen among those patients who received the pulses of GCs in the first 24 h (a) with respect to those who at that time did not receive it (b) is significantly greater.

In the case of a decrease in the concentration of ferritin, with a confidence level of at least 90%, we have no reason to conclude that there was a significant difference in the percentage of patients with decreasing ferritin in groups (a) vs. (b); however, it is observed that the maximum difference applies to patients from (e) and (g).

When comparing the groups of patients who were given (c) and who were not given (d) GCs-hd in the first 3 days, there are no significant percentage differences, while in the case of groups of patients who were given GCs-hd with IVIG, CyA (e) and patients who were given other, but different from the GCs-hd interventions (g), with 90% confidence, it can be concluded that the application for the first 3 days of the pulse of GCs with IVIG and CyA significantly increases the chances of an increase in fibrinogen compared to patients who did not receive the pulse of GCs in the first 3 days.

Table II. Characteristics of patients

Patients	Age [years]	sHLH subtype	Possible trigger	Clinical symptoms
1	11	PV-B19-HLH	PV-B19	Fever, hepatosplenomegaly, subcutaneous edema, erythema, interstitial pneumonitis, serositis, microhematuria
2	2	so-JIA-HLH	Unknown	Fever, hepatosplenomegaly, arthritis, rash
3	11	so-JIA-HLH	EBV	Fever, hepatosplenomegaly, lymphadenopathy, rash, arthritis, seizures, multi-organ dysfunction
4	5	UE-HLH	Unknown	Fever, hepatosplenomegaly, rash, pericarditis, palmar erythema
5	10	UE-HLH	Unknown	Fever, hepatosplenomegaly, rash, serositis, pneumonitis, respiratory failure
6	6	so-JIA-HLH	RV	Fever, hepatosplenomegaly, hemorrhagic rash, diarrhea
7	6	UE-HLH	Unknown	Fever, hepatosplenomegaly, arthritis, bradycardia
8	14	so-JIA-HLH	*Streptococcus viridans*	Fever, hepatosplenomegaly, subcutaneous edema, aphthous stomatitis, arthritis, seizures, brain edema, interstitial pneumonitis
9	1	UE-HLH	Unknown	Fever, hepatosplenomegaly, livedo reticularis, rash, renal failure
10	9	EBV-HLH	EBV	Fever, hepatosplenomegaly, hemorrhagic rash, lymphadenopathy, serositis, arthritis
11	7	so-JIA-HLH	EBV	Fever, splenomegaly, arthritis, pneumonia, generalized rash, palmar abscess
12	15	SLE-HLH	*Mycobacterium tuberculosis*	Fever, hepatosplenomegaly, lymphadenopathy, malar rash, proteinuria, serositis
13	12	Borrelia-HLH	*Borrelia burgdorferi*	Fever, hepatosplenomegaly, headache
14	9	so-JIA-HLH	*Mycoplasma*	Fever, splenomegaly, arthritis, rash, lymphadenopathy
15	10	so-JIA-HLH	*Yersinia*	Fever, hepatosplenomegaly, abdominal lymphadenopathy, generalized rash
16	17	Mycobacterium-HLH	*Mycobacterium xenopi*	Fever, hepatomegaly, peripheral edema, arrhythmia, interstitial pneumonitis, heart and respiratory failure, cholecystitis
17	8	so-JIA-HLH	CMV	Fever, hepatosplenomegaly, generalized erythema, lymphadenopathy, polyneuropathy, haemoptysis, multi-organ dysfunction
18	2	so-JIA-HLH	*Staphylococcus epidermidis*	Fever, hepatosplenomegaly, lymphadenopathy, serositis, conjunctivitis
19	16	so-JIA-HLH	CMV	Fever, hepatosplenomegaly, rash, myositis, serositis
20	6	so-JIA-HLH	Etanercept	Fever, arthritis, stomatitis, post reversible encephalopathy syndrome
21	14	UE-HLH	Unknown	Fever, rash, lymphadenopathy, pneumonitis
22	6	UE-HLH	EBV	Fever, hepatosplenomegaly, lymphadenopathy, pneumonitis, pleuritis, seizures, encephalitis
23	2	KD-HLH	Unknown	Fever, hepatosplenomegaly, lymphadenopathy, peripheral edema, palmar erythema, conjunctivitis, red, cracked lips
24	3	so-JIA-HLH	Unknown	Fever, splenomegaly, lymphadenopathy, interstitial pneumonitis, conjunctivitis, arthritis

sHLH – secondary hemophagocytic lymphohistiocytosis, EBV – Epstein-Barr virus, CMV – cytomegalovirus, PV-B19 – Parvovirus B19, so-JIA-HLH – systemic-onset juvenile idiopathic arthritis-associated HLH, UE-HLH – HLH of unknown etiology, SLE-HLH – systemic lupus erythematosus-associated HLH, KD-HLH – Kawasaki disease-associated HLH.

With a confidence level of 95%, it can be concluded that administration of GCs-hd on the first day or in the first three days from sHLH/MAS diagnosis has a favorable effect on the rapid resolution of fever and hepatomegaly. With respect to splenomegaly, this confidence can be placed at the

level of 90%. Additionally, in the case of patients who had a pulse of GCs in the first 3 days (c) with respect to those who did not have this intervention at that time (d), this conclusion may be given at a higher level of confidence of 95%.

Discussion

There are no exact data on the incidence of any form of acquired HLH. Our approximately 9-year-long observation suggests that the majority of children (58%) hospitalized for severe sHLH/MAS may suffer from autoimmune disease (both known and unknown at the time of sHLH/MAS diagnosis).

The choice of initial HLH treatment is difficult for several reasons. Some authors stress the need for aggressive treatment of HLH at the diagnosis, because the initial period of this syndrome is burdened with multi-organ complications and higher mortality [20–22]. Moreover, selection of optimal therapeutic interventions is difficult due to the inability to determine the cause of HLH and exclude genetically conditioned states (diagnostic and methodical limitations) in the early days of the disease. Secondary HLH is most often characterized by such features as age > 2 years, symptoms characteristic of systemic diseases, malignancies, circulatory failure symptoms, elevated levels of CRP, procalcitonin, CD163, increased number of leukocytes with a left shift, ferritin concentration > 10,000, no signs of acute liver failure, changes in the central nervous system, and hypogammaglobulinemia [23–28].

In the case of MAS, Ravelli *et al*. offered separate criteria for children with sJIA; however, in clinical practice, the HLH-2004 criteria proposed by the Histiocyte Society are commonly accepted [24, 29, 30]. In a recent paper, Davi *et al.* reported that HLH-2004 guidelines are likely not appropriate for identification of MAS in children with sJIA, because preliminary MAS guidelines showed the strongest ability to identify MAS in sJIA [31]. Currently, efforts are being made to establish the criteria for other forms of MAS, among others those based on the international consensus from 2011 [15]. The immunochemotherapy protocol proposed by the HLH Study Group is intended primarily for genetically conditioned states, and the authors point out that the treatment of sHLH should be targeted at treatment of the underlying condition [5]. However, when necessary (e.g., patients with recurrent or treatment-resistant HLH) the HLH-2004 protocol should be applied. In the case of a clinical suspicion of HLH, it is acceptable to start treatment before the patient meets the HLH-2004 criteria for diagnosis [5]. The results presented in this study confirm the belief, widely accepted in clinical practice (especially rheumatological), that patients with sHLH/MAS usually do not require the use of chemotherapy; in immunosuppression treatment, the main role is played by glucocorticoids and CyA [24, 30, 32, 33]. There are no conduct guidelines that relate to the doses of drugs and indications to extend treatment. Commonly applied treatment is based on the opinions of experts, case descriptions, and the personal experiences of given centers; it remains a matter that is often controversial. It is believed that minimal immunosuppression should be applied, which, on one hand, will control inflammation, and, on the other, minimize the risk of infectious complications during treatment, which constitute one of the main causes of death among patients with HLH. High doses of GCs are recommended by most authors as a first line of treatment; according to others, the first-line treatment is CyA [12, 14, 25, 32, 34–37]. Still others emphasize the crucial importance of IVIG, especially in the initial phase of treatment, increasing the concentration of ferritin [36]. Combined therapies are also common, for which indications are determined in each case individually.

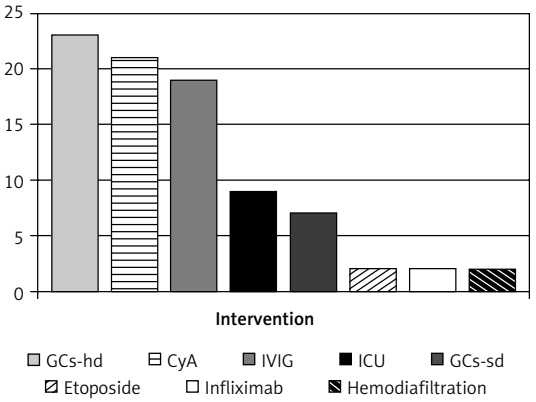

Figure 1. Administration frequency of different interventions in the studied sHLH/MAS patients

sHLH/MAS – secondary hemophagocytic lymphohistiocytosis/macrophage activation syndrome, GCs-hd – high-dose glucocorticoids, GCs-sd – standard-dose glucocorticoids, CyA – cyclosporine A, IVIG – intravenous immunoglobulins, ICU – intensive care unit.

The inflammatory cytokines are considered to play a key role in the pathogenesis of MAS. Successful treatment of severe pediatric rheumatic disease-associated MAS with interleukin-1 inhibition (anakinra) following conventional immunosuppressive therapy has been recently observed in a case series study [38]. Tumor necrosis factor (TNF) is considered a major proinflammatory cytokine, affecting various aspects of the immune reaction [39]. TNF-α inhibitors have demonstrated efficacy in patients with MAS who were refractory to conventional therapy [40]. In our study, infliximab was administered with good clinical response. However, it must be mentioned that

Table III. Therapeutic interventions used after sHLH/MAS diagnosis and analyzed time points T1 and T2 for the individual patient

Patients	Year of sHLH/MAS diagnosis	Therapies used in the first 72 h after sHLH/MAS diagnosis	Number of the day of commencement of a given therapy, counted from the time of sHLH/MAS diagnosis									
			1	2	3	4	5	6	7	8	9	> 9
1	2013	GCs-hd + IVIG + CyA	GCs-hd	IVIG + CyA		T1				T2		
4	2013	GCs-hd + IVIG + CyA	GCs-hd + IVIG + CyA		T1				T2			
7	2010	GCs-hd + IVIG + CyA	IVIG	GCs-hd + CyA		T1				T2		
15	2012	GCs-hd + IVIG + CyA	GCs-hd + CyA	IVIG		T1				T2		
24	2013	GCs-hd + IVIG + CyA	GCs-hd + CyA	IVIG		T1				T2		34: INFX
8	2013	GCs-hd + IVIG + CyA + ICU	GCs-hd + IVIG + CyA	ICU		T1				T2		
10	2012	GCs-hd + IVIG + CyA + ICU	GCs-hd + IVIG + CyA	ICU		T1				T2		
21	2013	GCs-hd + IVIG	IVIG	GCs-hd		T1				T2		
2	2012	GCs-hd + IVIG + other (GCs-sd)	GCs-sd + IVIG		GCs-hd		T1		CyA		T2	
5	2005	GCs-hd + IVIG + other (ICU)	GCs-hd	IVIG + ICU		T1				T2		15: CyA
6	2009	GCs-hd + IVIG	IVIG	GCS-hd		T1				T2		20: CyA
12	2012	GCs-hd + CyA	GCs-hd + CyA		T1				T2			
18	2007	GCs-hd + CyA	GCs-hd + CyA		T1				T2			
20	2013	GCs-hd + CyA	GCs-hd + CyA		T1						T2	
22	2010	GCs-hd + CyA	GCs-hd + CyA		T1	ICU		IVIG		T2		13: VP16
3	2009	GCs-hd + CyA + other (GCs-sd, ICU)	GCs-sd + CyA	GCs-hd + ICU	VP-16		T1	HF		T2	IVIG + INFX	
9	2012	Other (IVIG)	IVIG		T1	GCs-hd	ICU		CyA		T2	

Table III. Cont.

Patients	Year of sHLH/MAS diagnosis	Therapies used in the first 72 h after sHLH/MAS diagnosis	Number of the day of commencement of a given therapy, counted from the time of sHLH/MAS diagnosis									
			1	2	3	4	5	6	7	8	9	> 9
11	2012	Other (GCs-sd, IVIG, CyA)	GCs-sd + IVIG + CyA		T1	GCs-hd		T2				
13	2007	Other (CyA)	CyA		T1				T2	GCs-hd		
14	2006	Other (GCs-sd)	GCs-sd		IVIG		T1				T2	13: GCs-hd + CyA
16	2013	Other (GCs-sd, IVIG, CyA, ICU)	GCs-sd + IVIG	CyA	ICU		T1		GCs-hd		T2	
17	2006	Other (GCs-sd)	GCs-sd		IVIG		ICU		GCs-hd		T2	12: HF
19	2007	Other (GCs-sd)	GCs-sd		T1				T2	CyA		
23	2010	Other (GCs-sd)	GCs-hd		T1			IVIG		T2		

GCs-sd – low-dose glucocorticoids, GCs-hd – high-dose glucocorticoids, IVIG – intravenous immunoglobulins, CyA – cyclosporine A, VP16 – etoposide, INFX – infliximab, ICU – intensive care unit, HF – hemofiltration

patients on TNF-α inhibitors (infliximab, adalimumab, etanercept) are at higher risk of infections, e.g. reactivation of latent tuberculosis [41]. Other potential side effects, including malignancies, have also been observed after therapy with TNF-α inhibitors. Nevertheless, there are conflicting data on this issue without convincing evidence for increased risk of lymphoma or solid tumors solely due to the anti-TNF therapy [39, 42]. Moreover, some case reports have postulated the development of MAS after initiation of TNF-α inhibitors in patients with rheumatic diseases as a possible result of the cytokine dysregulation [43]. However, there is no reliable evidence supporting this hypothesis, since it could be the natural course of the disease and a coincidence with onset of the therapy. Further prospective studies on this approach are required to better define the role of biological agents in the management of MAS.

In the present study, the basic methodology was a combined treatment: pulses of methylprednisolone ± IVIG +/and CyA, although the selection of optimal therapy was in each case individualized. The delay in implementation of GCs in some children was usually associated with the need to carry out diagnostics, which would be difficult after administration of GCs (histopathological examination of bone marrow and tissue). In cases of a severe, life-threatening condition, GC treatment was started at the time of diagnosis. Simultaneously, all the children had anti-bacterial and anti-fungal treatment (empirical), focused on the potential cause of HLH, as well as prevention of infections. We did not observe secondary infection as an iatrogenic complication of the treatment.

All of the patients survived; hence in this study we examined selected clinical parameters that, according to the work of other authors, are clinically important prognostic factors (endpoints) for patients with HLH (i.e., fever, splenomegaly, concentrations of ferritin, fibrinogen, bilirubin, albumin, hemoglobin, and thrombocytes) [44–47].

In the present study over one third of patients required supportive treatment in the ICU (38%). These results are similar to others recently reported [48]. The reason for treatment in intensive care in all cases was worsening of cardiopulmonary insufficiency, which was probably related to involvement of heart muscle (inflammation of the pericardium and/or myocardium) or lungs (interstitial infiltrates). We believe that the improvement or deterioration in the analyzed laboratory parameters was not related directly to cardiopulmonary and respiratory failure or hospitalization in the ICU.

The results we report support the benefits of combined sHLH/MAS therapy and early implementation of GCs in high doses – faster resolution of fever, hepatosplenomegaly, and the rise of

Table IV. Results of the studied variables analyzed at time points T1 and T2

Patients	Therapy applied in the first 72 h after diagnosis	PLT		WBC		HGB		FBG		FERR	
		T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
1	GCs-hd + IVIG + CyA	250	501	10.0	6.9	11.0	10.8	1.6	1.9	8000	2097
2	GCs-sd + IVIG + GCs-hd + (CyA)	420	704	18.6	25.7	11.2	11.4	1.48	1.1	2601	2885
3	GCs-sd + GCs-hd + CyA + VP16 + ICU + HF	21	9	3.3	2.0	9.0	9.6	0.9	0.67	57000	16850
4	GCs-hd + IVIG + CyA	65	290	16.2	10.0	10.2	11.1	0.48	0.97	5024	2403
5	GCs-hd + IVIG + ICU	83	444					2.7	1.2	4576	7854
6	GCs-hd + CyA	308	291	3.5	5.7	9.6	8.6	3	2.3	1525	1143
7	GCs-hd + IVIG + CyA	120	370					1.5	3	6000	869
8	GCs-hd + IVIG + CyA + ICU	166	300	3.0	12.0	8.7	10.2	1.8	3	60000	3756
9	IVIG + (GCs-hd + ICU + CyA)	290	78	17.0	21.0	8.5	10.3	3.65	0.59	2676	2643
10	GCs-hd + IVIG + CyA + ICU	108	245	7.8	3.5	10.3	10.9	0.53	0.67	73456	4804
11	GCs-sd + IVIG + CyA + (GCs-hd)	139	246	16.9	22.0	9.2	11.1	0.5	2.3	5024	12500
12	GCs-hd + CyA	60	285	5.6	10.7	9.5	11.0	1.9	2.6	10785	2000
13	CyA	206	462	6.4	8.0	10.2	10.7	4.6	6.2	576	246
14	GCs-sd + IVIG	125	195		8.0	9.5	11.0	2.4	2.6	3576	2600
15	GCs-hd + IVIG + CyA	140	370					2.2	1.2	40000	12000
16	GCs-sd + IVIG + CyA + ICU + (GCs-hd)	73	159	2.7	4.0	10.6		1.8	1.6	6462	5464
17	GCs-sd + IVIG + ICU + (GCs-hd)	327	15	6.5	6.0	8.6	11.4	2	0.8	2000	3245
18	GCs-hd + CyA	288	789	7.2	19.8	10.8	10.7	2	3	16578	5867
19	GCs-sd	280	300		18.0		10	8	7	5000	4000
20	GCs-hd + CyA + (ICU)	271	350	7.9		11.4		1.8	1.6	4000	1400
21	IVIG + GCs-hd	72	374	4.0	14.1	10.5	11.2	0.8	2.0	8000	9546
22	GCs-hd + CyA + (ICU + IVIG)	220	407	41.0	26.0	13		1.04	4	15980	6480
23	GCs-hd + (IVIG)	947	405	28.0	28.0	7.7	9.2	3	4	2700	4200
24	GCs-hd + IVIG + CyA	66	150	5.4		10.1	9.7	0.68	2.0	31189	14056

GCs-sd – low-dose glucocorticoids, GCs-hd – high-dose glucocorticoids, IVIG – intravenous immunoglobulins, CyA – cyclosporine A, VP16 – etoposide, INFX – infliximab, ICU – intensive care unit, HF – hemofiltration, PLT – platelets, WBC – white blood cells, HGB – hemoglobin, FBG – fibrinogen, FERR – ferritin.

Table V. Comparison of behavior of HLH parameters (ferritin, platelets, fibrinogen, duration of fever, hepatomegaly and splenomegaly) with three established divisions of test groups

	GCs-hd intervention in the first 24 h after sHLH/MAS diagnosis		GCs-hd intervention in the first 72 h after sHLH/MAS diagnosis		Intervention in the first 72 h after sHLH/MAS diagnosis		
	Yes (*n* = 12, 100%) (a)	No (*n* = 12, 100%) (b)	Yes (*n* = 17, 100%) (c)	No (*n* = 7, 100%) (d)	GCs-hd IVIG CyA (*n* = 7, 100%) (e)	GCs-hd CyA (*n* = 5, 100%) (f)	Others, but different to GCs-hd (*n* = 7, 100%) (g)
Decrease of ferritin:							
% persons	83.3	66.7	76.5	71.4	100	100	62.5
Min-max[1]	52.2–93.7	1.2–85.5	25.0–93.7	1.2–57.3	52.2–93.7	59.4–81.5	1.2–57.3
Mean ± SD	70.9 ±14.8	37.8 ±29.6	68.4 ±18.7	24.2 ±20.8	74.8 ±17.1	68.2 ±8.4	24.2 ±20.8
Median	67.5	26.1	70.0	20.0	73.8	65.0	20.0
Increased platelet count:							
% persons	91.7	66.7	82.4	71.4	100	80	75
Min-max	29.2–434.9	7.1–419.4	29.2–434.9	7.1–124.3	80.7–346.2	29.2–375.0	7.1–419.4
Mean ± SD	185.8 ±135.68	134.7 ±129.4	195.7 ±139.2	76.4 ±48.0	164.9 ±90.2	165.8 ±151.7	133.6 ±146.5
Median	127.3	97.4	145.8	77.0	127.3	127.3	97.4
Increase in fibrinogen:							
% persons	75.0	41.7	64.7	42.9	85.7	60.0	50.0
Min-max	18.7–284.6	8.3–360.0	18.7–284.6	8.3–360.0	18.7–194.1	36.8–284.6	8.3–360.0
Mean ± SD	90.3 ±90.9	130.6 ±139.7	96.6 ±83.3	134.4 ±195.8	84.7 ±64.2	123.8 ±139.4	138.3 ±160.1
Median	50.0	100.0	66.7	34.8	83.3	50.0	92.4
Number of days to abatement of fever:							
Min–max	0–8	1–15	0–8	1–15	1–2	0–1	1–15
Mean ± SD	1.7 ±2.1	4.2 ±4.3	1.6 ±1.8	6.1 ±4.8	1.1 ±0.4	0.8 ±0.5	5.6 ±4.7
Median	1	2	1	7	1	1	5
Percentage of cases with a given number of days to abatement of hepatomegaly:							
0	8.3	16.7	11.8	14.3	0	20.0	25.0
1–7	33.3	16.7	35.3	0	42.9	0	0
8–14	50.0	8.3	41.2	0	57.1	60.0	0
> 14	8.3	58.3	11.8	85.7	0	20.0	75.0
Percentage of cases with a given number of days to abatement of splenomegaly:							
0	25.0	25.0	29.4	14.3	14.3	40.0	25.0
1–7	66.7	41.7	64.7	28.6	71.4	60.0	25.0
8–14	8.3	25.0	5.9	42.9	14.3	0	37.5
> 14	0	8.3	0	14.3	0	0	12.5

n – number, SD – standard deviation, Min – minimum value, Max – maximum value, sHLH/MAS – secondary hemophagocytic lymphohistiocytosis/macrophage activation syndrome, GCs-hd – high-dose glucocorticoids, IVIG – intravenous immunoglobulins, CyA – cyclosporine A.

[1]Range, average, SD and median for the variable describing how far, as a percentage, the value of ferritin decreased between T1 and T2, similarly for the other two growth parameters

Table VI. Division of confidence levels into levels of confidence of 95% and 90% for the difference of patients, respectively, with the decline of ferritin, and increase in thrombocytes and fibrinogen

	(a) vs. (b)	(c) vs. (d)	(e) vs. (g)
Decrease of ferritin	[–0.14; 1.00] [–0.10; 1.00]	[–0.26; 1.00] [–0.21; 1.00]	[–0.09; 1.00] [–0.003;1.00]
Increase in thrombocytes	[–0.04; 1.00] **[0.02; 1.00]**	[–0.19; 1.00] [–0.13; 1.00]	[–0.09; 1.00] [–0.003;1.00]
Increase in fibrinogen	[–0.04; 1.00] **[0.03; 1.00]**	[–0.17; 1.00] [–0.08; 1.00]	[–0.08; 1.00] **[0.02; 1.00]**

a) high-dose glucocorticoids administered ≤ 24 h after diagnosis of hemophagocytic lymphohistiocytosis (HLH), b) high-dose glucocorticoids administered > 24 h after diagnosis of HLH, c) high-dose glucocorticoids administered ≤ 72 h after diagnosis of HLH, d) high-dose glucocorticoids administered > 72 h after diagnosis of HLH, e) high-dose glucocorticoids administered together with intravenous immunoglobulins and cyclosporine A, f) high-dose glucocorticoids administered together with cyclosporine A, g) therapy other than high-dose glucocorticoids.

Table VII. Division of confidence levels into levels of confidence of 95% and 90% for the difference of patients, respectively, for a time of abatement of hepatomegaly > 14 days and for a time of abatement of splenomegaly > 14 days

	(a) vs. (b)	(c) vs. (d)	(e) vs. (g)
Hepatomegaly > 14 days	**[–1.00; –0.18]** **[–1.00; –0.24]**	**[–1.00; –0.36]** **[–1.00; –0.44]**	**[–1.00; –0.41]** [–1.00; –0.49]
Splenomegaly > 14 days	[–1.00; 0.04] **[–1.00; –0.02]**	**[–1.00; –0.16]** **[–1.00; –0.22]**	[–1.00; 0.22] **[–1.00; –0.02]**

a) high-dose glucocorticoids administered ≤ 24 h after diagnosis of hemophagocytic lymphohistiocytosis (HLH), b) high-dose glucocorticoids administered > 24 h after diagnosis of HLH, c) high-dose glucocorticoids administered ≤ 72 h after diagnosis of HLH, d) high-dose glucocorticoids administered > 72 h after diagnosis of HLH, e) high-dose glucocorticoids administered together with intravenous immunoglobulins and cyclosporine A, f) high-dose glucocorticoids administered together with cyclosporine A, g) therapy other than high-dose glucocorticoids.

fibrinogen concentration and platelet count. No statistically significant differences were found for the combination of associated therapies with respect to the concentration of ferritin.

We are aware that the study has certain limitations. It is a retrospective population-based analysis from a single center, the group of patients was heterogeneous, and analyzed parameters represent substitute points. We used statistical methods based on construction of the smallest one-sided confidence interval for the difference of two proportions – a method with limited statistical power. Due to the number of comparisons, statistical differences may have been observed by chance. The study was not randomized and the therapy was not standardized. Since the treatment must start from the early beginning of the disease, implementation of a standardized scheme is always very difficult and raises ethical doubts. The treatment was individualized, depending on the patient's clinical condition. Determination of the impact of a given intervention on the clinical course and laboratory test results was one of the aims of the study. Furthermore, several other factors not analyzed in this study can influence the course of HLH. Of significance may be the dosage of drugs used (range of the pulse dose: 10–30 mg/kg/day), individual metabolism of the drugs (oxygen saturation time, therapeutic dose), interactions between them, and innate immune mechanisms. Finally, the effect evaluated after seven days from the last intervention applied in the first 72 h from diagnosis is the sum of the effects, which may also be influenced by interventions not included in the analysis, i.e., antibiotic therapy, blood preparations, nutritional or symptomatic therapy.

However, we are unaware of any studies that answer the question what is the influence of particular HLH therapy on clinical symptoms and laboratory markers. We believe that inappropriate treatment such as immunosuppression monotherapy and a delay in the start of treatment may be one of the main unfavorable prognostic factors in patients with HLH. Our main goal was to underline the positive effect of combined immunosuppression as the initial therapy for patients with sHLH.

Further analysis is required. A well-designed, prospective cohort study would provide the strongest scientific evidence.

In conclusion, in the treatment of sHLH/MAS administration of high-dose glucocorticoids, CyA, and IVIG is crucial for a successful outcome. Usually, there is no need for additional chemotherapy. The application of high-dose GCs in the first 24 h or high-dose GCs in combination with CyA and IVIG in the first 72 h after diagnosis have a positive influence on the improvement in terms of disease markers such as thrombocytes, fibrinogen concen-

tration and the alleviation of fever, splenomegaly and hepatomegaly in children with sHLH/MAS.

In recent years, it has been stressed that sHLH/MAS is a polygenic condition [19, 24]. The clinical course and prognosis may therefore depend not only on intervention but also on the efficiency of the immune mechanisms involved in the pathogenesis of HLH. Further research is essential, aimed at identifying these pathomechanisms, expanding diagnostic methods in patients with HLH by detailed genetic and functional studies, as they can allow for a more accurate understanding of the essence of HLH and carrying out studies evaluating the impact of commonly used therapeutic interventions ("classic" drugs and immunosuppressive methods vs. chemotherapy) on the clinical course and the choice of optimal treatment.

Conflict of interest

The authors declare no conflict of interest.

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