Histiocyte Society

HLH-2004

Hemophagocytic Lymphohistiocytosis Study Group

Treatment Protocol of the Second International HLH Study 2004

Start of the Study: January 2004

Chairman: Jan-Inge Henter, M.D., Ph.D., Stockholm, Sweden

CONTENTS

STUDY COMMITTEE

Study Chairman

Jan-Inge Henter, MD, PhD
Child Cancer Research Unit
Department of Pediatrics
Karolinska Hospital, Q6:05
S-171 76 Stockholm, Sweden
Tel: +46 - 8 5177 2870 (secr),
Fax: +46 - 8 5177 3184
E: Jan-Inge.Henter@kbh.ki.se

Study Coordinator

AnnaCarin Horne, MD
Child Cancer Research Unit
Department of Pediatrics
Karolinska Hospital, Q6:05
S-171 76 Stockholm, Sweden
Tel: +46 - 8 5177 7098 (office)
Fax: +46 - 8 5177 3184
E: ACHorne@telia.com

Clinical Study Group

Maurizio Aricò, MD
Oncoematologia Pediatrica
Ospedale dei Bambini
90100 Palermo, Italy
Tel: +39 - 091-6666-131
Fax: +39 - 091-6666-001 (202)
E: arico@ospedalecivicopa.org

R Maarten Egeler, MD, PhD
Dept of Pediatrics, Rm J6-222
Leiden University Medical Center
PO Box 9600
2300 RC Leiden, The Netherlands
Tel: +31 - 71 526-4131/2824
Fax: +31 - 71 524-8198
E-mail: RM.Egeler@LUMC.nl

Lisa H Filipovich, MD
Children´s Hosp Medical Center
Div of Hematology/Oncology
3333 Burnet Avenue
Cincinnati, Ohio 45229, USA
Tel: +1 - 513 636-7206
Fax: +1 - 513 636-5845
E: Lisa.Filipovich@chmcc.org

Shinsaku Imashuku, MD,
Dir, Kyoto City Inst of Health
and Environmental Sciences
1-2 Higashitakada-cho, Mibu
Nakagyo-ku, Kyoto, Japan 604
Tel: +81 - 75 312 4941
Fax: +81 - 75 311 3232
shinim95@mbox.kyoto-inet.or.jp

Prof Dr Gritta Janka-Schaub
Children's University Hospital
Dept of Ped Hematol Oncol
Martinistrasse 52
20246 Hamburg, Germany
Tel: +49 -40 42803-3796 (4270)
Fax: +49 - 40 42803-2580 (4601)
E: janka@uke.uni-hamburg.de

Stephan Ladisch, MD
Children's National Medical Cent
111 Michigan Avenue N.W.
Washington DC, 20010-2970
Tel: +1 - 202 884 3898
Fax: +1 - 202 884 3929
E: SLadisch@cnmc.org

Ken McClain, MD, PhD
Pediatric Hematology/Oncology
Texas Children's Hospital
CC 1510.00, 6621 Fannin St,
Houston, TX 77030, USA
Tel: +1 - 832-822-4208
Fax: +1 - 832-822-1503
E: kmcclain@txccc.org

The UK representative is to be announced. Meanwhile contact:
David Webb, MD
Dept of Haematology/Oncology
The Hospitals for Sick Children,
Great Ormond Street
London WC1N 3JH, England
Tel: + 44 - 207 405 9200
Fax: + 44 - 207 813 8410
E: David.Webb@gosh-tr.nthames.nhs.uk

BMT advisor

Jacek Winiarski, MD, PhD
Dept of Pediatrics
Huddinge University Hospital
S-141 86 Huddinge, Sweden
Tel: +46 - 8 5858 7336
Fax: +46 - 8 5858 7390
E: Jacek.Winiarski@klinvet.ki.se

Biology Study Advisors

Bengt Fadeel, MD, PhD
Inst of Environmental Medicine
Nobels väg 13, Karolinska Inst
S-171 77 Stockholm, Sweden
Tel: + 46 8 728 75 56
Fax: + 46 8 32 90 41
E: Bengt.Fadeel@imm.ki.se

Marion Schneider, PhD
Sektion Experimentelle
Anästhesiologie
Universitätsklinikum Ulm
Steinhövelstrasse 9
89075 Ulm, Germany
Tel: +49-731 500-27940 (7943)
Fax: +49-731 500-26755
E:marion.schneider@medizin.uni-ulm.de

LOCAL COORDINATORS

AUSTRIA:
Milen Minkov, MD
St Anna Children's Hospital
Kinderspitalgasse 6
A-1090 Vienna, Austria
Tel: +43 - 1 40 170 250
Fax: +43 - 1 40 170 430
E: Minkov@ccri.univie.ac.at

SPAIN:
Itziar Astigarraga, MD, PhD
Pediatric Oncology Unit
Hospital de Cruces
48903. Barakaldo. Vizcaya.
Spain
Tel: + 34 94 6006331
Fax: + 34 94 6006155
iastigarraga@hcru.osakidetza.net

SOUTH-AMERICA:
Jorge Braier, MD
Hem/Onc, Hospital Garrahan
Combate de los Pozos 1881
Buenos Aires 1245, Argentina
Tel:+54 11 43084 300
Fax:+54 11 4308 5325
E: jbraier@intramed.net.ar

STUDY DATAMANAGER

Martina Löfstedt
Childhood Cancer Research Unit
Karolinska Hospital, Q6:05
S-171 76 Stockholm, Sweden
Tel: +46 - 8 5177 7098 (2870)
Fax: +46 - 8 5177 3184
E: Martina.Lofstedt@ks.se

North-Am Registrations

Histiocyte Society
72 East Holly Ave Suite 101
Pitman, NJ 08071, USA
Tel: +1 856 589 6606
Fax: +1 856 589 6614
E: HSProtocols@aol.com

SENIOR ADVISORS

Göran Elinder, MD, PhD
Sachs' Children's Hospital
S-118 83 Stockholm, Sweden
Tel: +46 - 8 616 4048
Fax: +46 - 8 616 4110
E: goran.elinder@admin.ki.se

Prof Dr Helmut Gadner
St Anna Children's Hospital
Kinderspitalgasse 6
A-1090 Vienna, Austria
Tel: +43 - 1 40 170 250
Fax: +43 - 1 40 170 430
E: Gadner@ccri.univie.ac.at

Diane Komp, MD
Tel & Fax: +1 - 203 453 3821
E: DoktorDi@aol.com

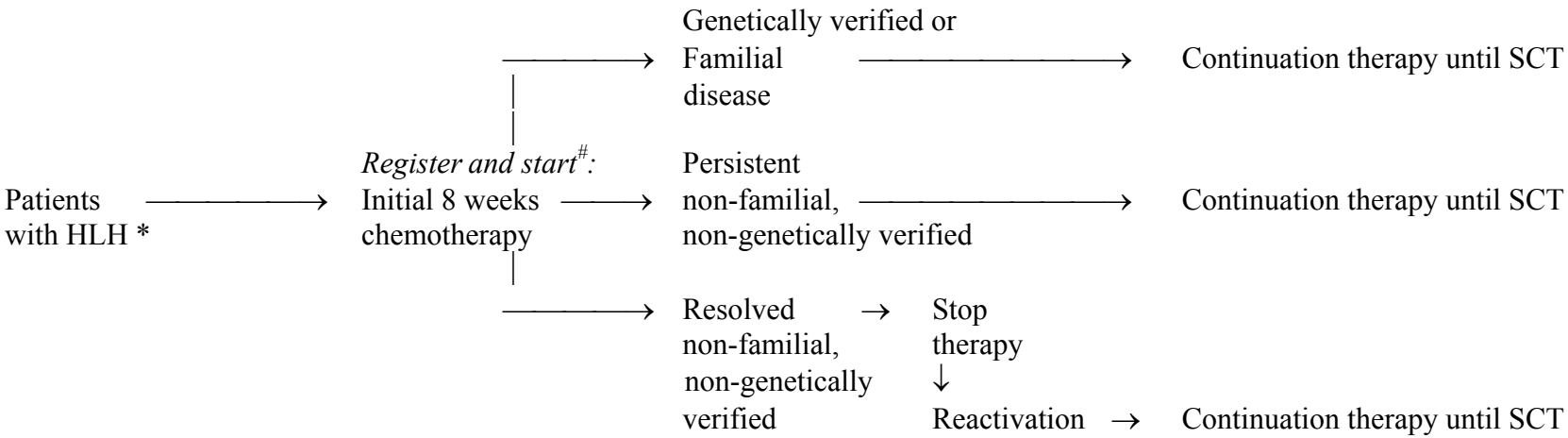
DRUG SAFETY MONITORING BOARD

CHAIRMAN:
Finn Wesenberg, MD, PhD
Department of Pediatrics
National Hospital
N-0027 Oslo, Norway
Tel: + 47 2307 3207
Fax: + 47 2307 4570
finn.wesenberg@rikshospitalet.no

Åke Jakobson, MD, PhD
Pediatric Hematology/Oncology
Karolinska Hospital, Q6:04
S-171 76 Stockholm, Sweden
Tel: +46 - 8 5177 9576
Fax: +46 - 8 5177 3184
E: ake.jakobson@ks.se

James Whitlock, MD
Vanderbilt Univ Medical Center
2220 Pierce Ave
397 PRB Pediatric Hem/Onc
Nashville, TN 37232-6310
Tel: +1 615 936 1762
Fax: +1 615 936 1767
E: jim.whitlock@Vanderbilt.Edu

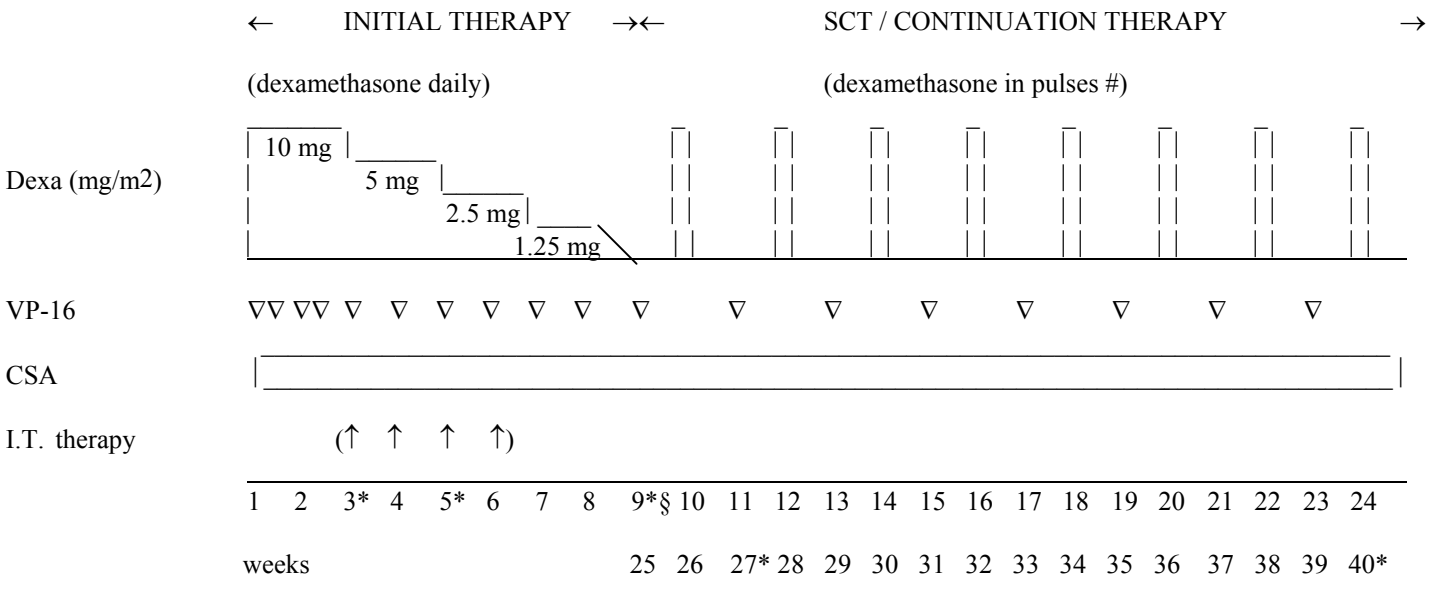
Figure 1: Flow-sheet for Children with Hemophagocytic Lymphohistiocytosis (HLH) in HLH-2004



* If there is a treatable infection it should be treated but be aware that this may not be sufficient and the patient may need HLH-treatment in addition. All severe forms should start HLH-treatment. If HLH is persistent or recurring consider that the patient may have an undiagnosed inherited disease. HLH may also develop secondary to a number of other diseases as malignancies, rheumatic diseases and metabolic disorders, requiring a different treatment.

Start therapy if the patient has a genetically verified disease, a familial form of HLH, or if the disease is severe, persistent, or recurrent.

Figure 2: Treatment protocol overview for Hemophagocytic Lymfohistiocytosis (HLH-2004)



Go to SCT during continuation therapy as soon as an acceptable donor is available with
- HLA-identical related donor or
- Matched unrelated donor or
- Mismatched unrelated donor or
- Family haploidentical donor

(further SCT information: see text)

A donor search as soon as possible is suggested in familial patients and poorly responding patients, and is to be considered in infants.

Doses calculated per m^2 also if BW <10kg

* = EVALUATION, see Table 1

§ See Fig 1 for info on start of Continuation

Dexa = Dexamethasone daily with 10 mg/m^2 for 2 weeks, 5 mg/m^2 for 2 weeks, 2.5 mg/m^2 for 2 weeks, 1.25 mg/m^2 for 1 week; and taper then discontinue during 8th week.
# Pulses every second week with 10 mg/m^2 for 3 days during the continuation therapy.

VP-16 = Etoposide 150 mg/m^2 i.v., twice weekly for the first two weeks, then weekly during the initial therapy. Every second week during the continuation therapy.
Only in certain conditions, such as if ANC <0.5 x10^9/L and the bone marrow is hypocellular (which only rarely is the case), can the first two doses be omitted.

CSA = Cyclosporin A aiming at levels around 200 microg/L (monoclonal, trough value). Start with 6 mg/kg daily orally (divide in 2 daily doses), if normal kidney function.

I.T. therapy : ↑ = Methotrexate doses by age: <1 year 6 mg, 1-2 years 8 mg, 2-3 years 10 mg, >3 years 12 mg each dose.
Prednisolon doses by age: <1 year 4 mg, 1-2 years 6 mg, 2-3 years 8 mg, >3 years 10 mg each dose.
Maximum four doses are suggested, but start only if progressive neurological symptoms or if an abnormal CSF has not improved.

Supportive therapy: Cotrimoxazole, eq 5 mg/kg of trimethoprim, 2-3 times weekly (week 1 and onwards). An oral antimycotic from week 1 to week 9.
IvIG (0.5 g/kg iv) q 4 weeks. Gastroprotection suggested week 1-9.

Figure 3. Documentation Sheet for the Initial Therapy in HLH-2004 (week 1-8)

(To be sent to local subcenter/coordinator, with Follow-Up Report Sheet 2 months after onset of therapy)

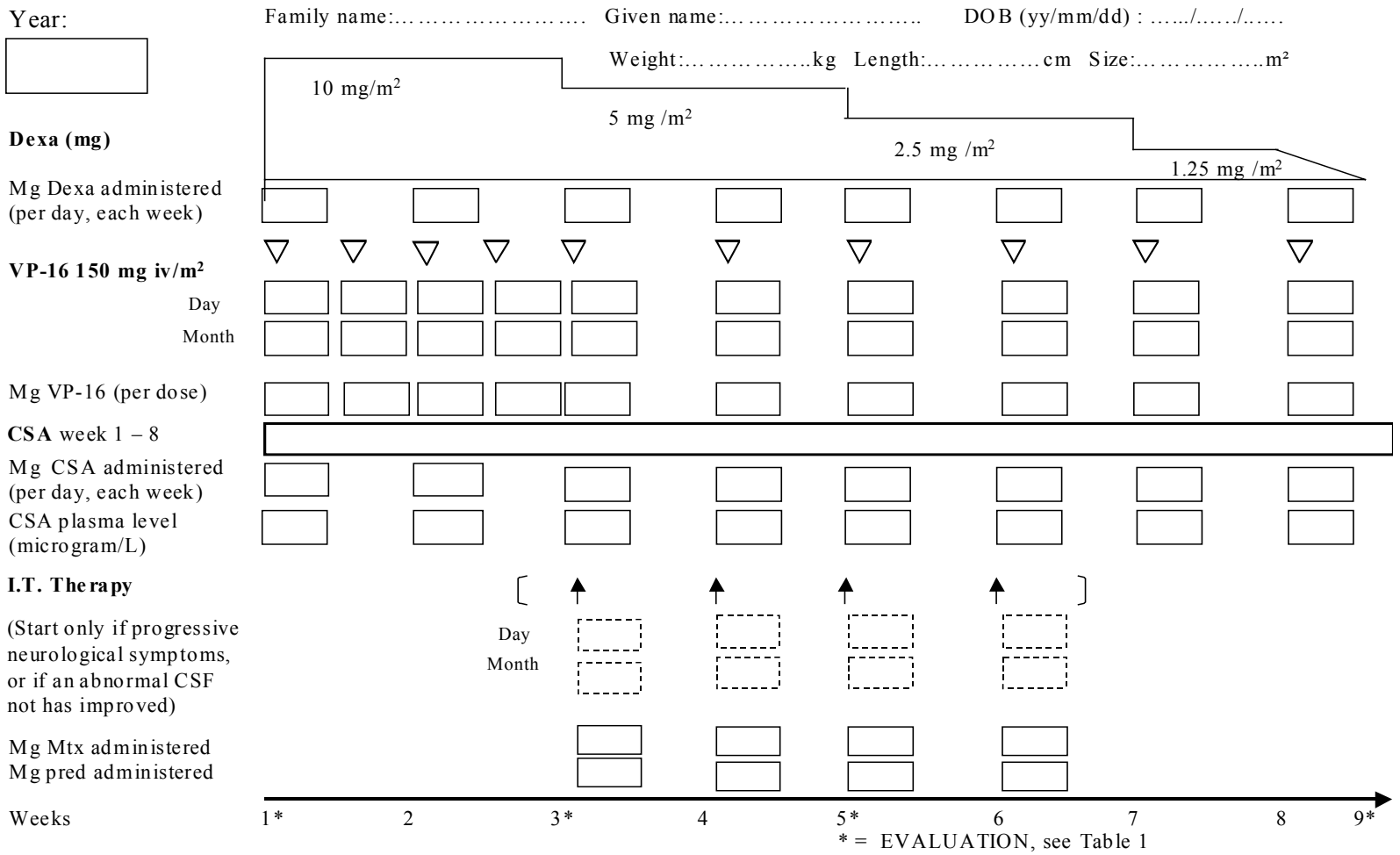


Figure 4. Documentation Sheet for the Continuation Therapy in HLH-2004 week 9-24

(To be sent to local subcenter/coordinator, with Follow-Up Report Sheet 6 months after onset of therapy)

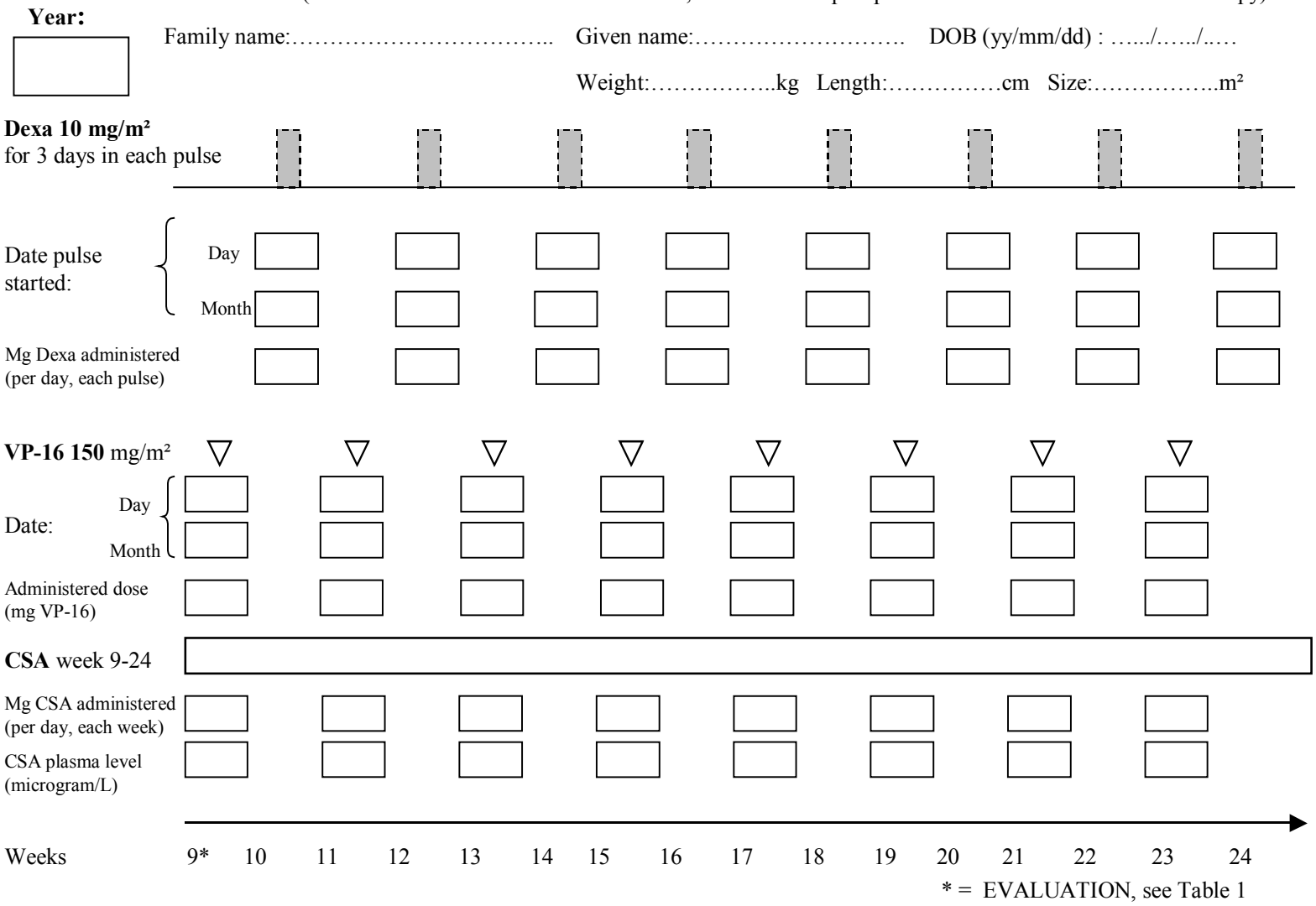


Figure 5. Documentation Sheet for the Continuation Therapy in HLH-2004 week 25-40

(To be sent to local subcenter/coordinator, with Follow-Up Report Sheet 12 months after onset of therapy)

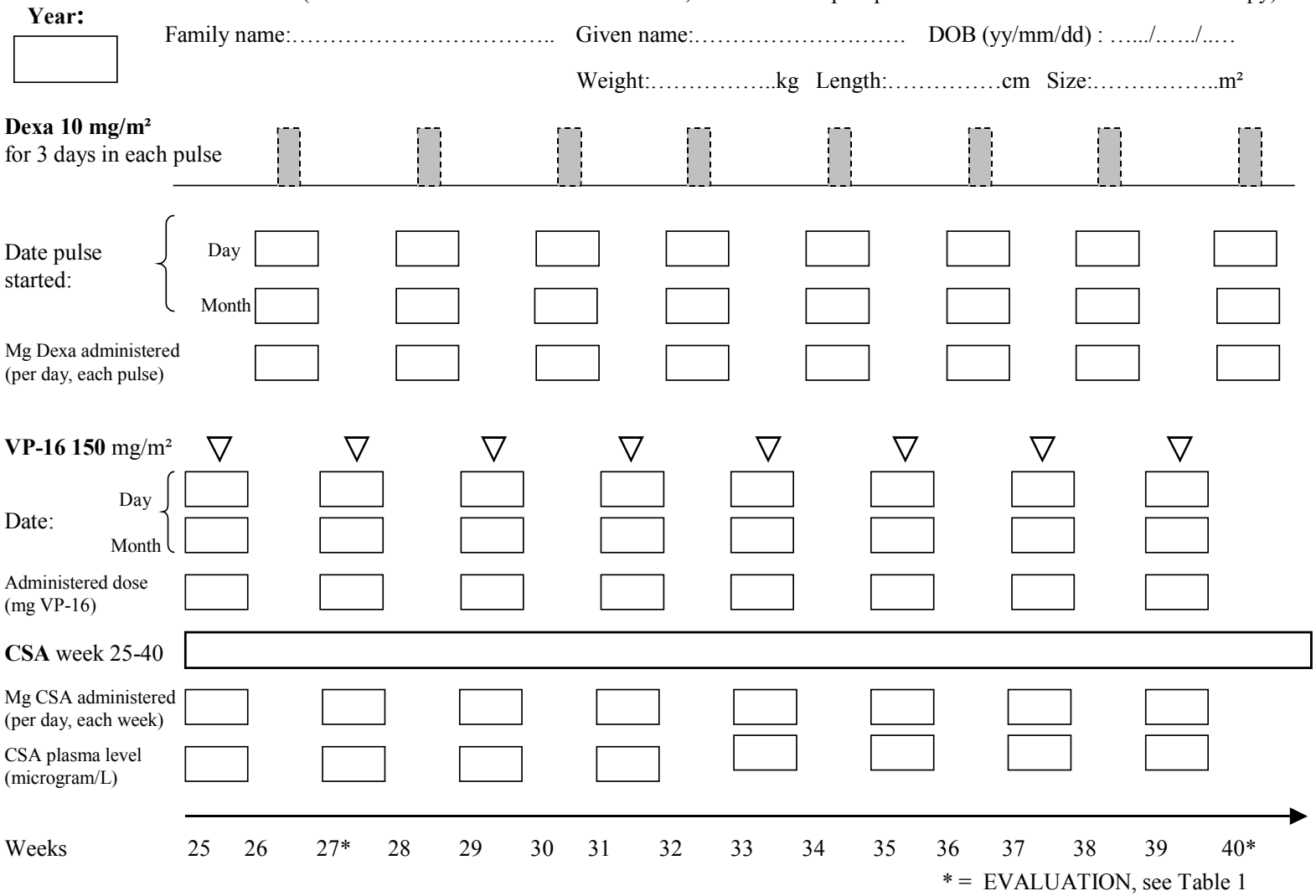


Table 1: Assessment for patients with HLH (in HLH-2004)

Required evaluations[1]	← weeks of treatment →																																→	Every 12 mo	If re-activ-ation	If CNS-activ-ation	Pre-SCT
	1	2	3	4	5	6	7	8	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39	41												
Hb, WBC, diff, platelets	← once weekly →								← recommended every second week →																											x	x	x	x
Ferritin, transaminases	← every second week →								← recommended every second week →																											x	x	x	x
Triglycerides, fibrinogen	← every second week →								x									x																	x[7]	x	x	x	x
Creatinine	← every second week →								← recommended every second week →																											x	x	x	x
CSA-levels[2]	← recommended initially weekly, later every second week →																																			x	x	x	x
APTT/PT/D-dimers	x (then follow as clinically indicated)																																				x	x	x
GFR	recommended as early as feasible in association with CSA-start, at least if creatinine is elevated, and later as indicated																																						
CSF (cells/protein)	x		(x	x	x	x)			(x)									(x)																	(x)[7]	(x)	x	x	x
Histology/cytology[3]	x																																				x	x	
Chest X-ray (or CT)	x								(x)									(x)																	(x)[7]	(x)	x	x	x
Abdominal ultrasound (or CT)	x								(x)									(x)																			x	x	x
MR of the brain	x								(x)									(x)																	(x)[7]	(x)	x	x	x
NK-cell activity[4]	x								(x)																														x
Genetic analyses[5]		x																																				
Soluble IL-2receptor (sCD25)[6]	(x)		(x)		(x)				(x)									(x)																	(x)[7]		(x)	(x)	(x)

1 **Additional pre-treatment investigations, with reticulocytes, serum electrolytes and, in particular, the infectious investigation, see page 20.**
HLA-typing as soon as feasible (see page 21).
Investigations in brackets are optional and are to be done only if there has been previous signs of involvement of the particular analysis.

2 Monoclonal antibody assay of whole blood.

3 Safe and available tissue, such as bone marrow, lymph node, liver or CSF

4 Specific HLH-2004-laboratories suggested (for addresses, see page Appendix-19)

5 Genetic analysis for perforin and hMunc gene defects or flow cytometry perforin screeening is recommended. Specific HLH-2004-laboratories suggested (see page App-19).

6 Soluble IL-2 receptor (sCD25) is optional, since it is not readily available, but suggested if available. As a voluntary parameter, it may analyzed more frequently.

7 The additional examinations after 40 weeks intended for patients stopping therapy at this point.

GENERAL BACKGROUND

Nomenclature

The term histiocytoses identifies a group of disorders that have in common the proliferation of cells of the mononuclear phagocyte system, with the histiocyte as a central cell. The histiocytic disorders are, at present, in brief classified as follows (1):

DISORDERS OF VARIED BIOLOGICAL BEHAVIOR
- *Dendritic cell-related disorders (incl Langerhans cell histiocytosis)*
- *Macrophage-related disorders*

MALIGNANT DISORDERS

Hemophagocytic lymphohistiocytosis (HLH) includes the great majority of patients with macrophage-related disorders (1-5). HLH comprises two different conditions that may be difficult to distinguish from each other (4-7):

(i) *Familial hemophagocytic lymphohistiocytosis (FHL)* (primary HLH)
FHL is an autosomal recessive disease, in some patients associated with decreased apoptosis triggering (8). One of the underlying gene defects can be mutations in the perforin gene (9-15), which account for 20-40% of all affected FHL families (10). This causes a defect in NK- and T cell cytotoxicity (12, 16-22). Mutations in the gene hMunc 13-4, essential for cytolytic granules fusion, may also cause FHL (23). Despite the name, *familial hemophagocytic (erythrophagocytic) lymphohistiocytosis (FHL or FEL)*, the family history is often negative, since the disease is recessive. The onset of FHL and bouts of the disease may be triggered by infections. The incidence of FHL has (in Sweden) been estimated to 1.2/1,000,000 children/year (around 1:50,000 live born) (24).

(ii) *Secondary hemophagocytic syndrome* (secondary HLH, sHLH)
A macrophage activation syndrome (MAS) with hemophagocytosis may develop also as a result of a strong immunological activation of the mononuclear phagocyte system, such as a severe infection. The condition has been described in immuno-compromised hosts in association with viral infections and the term *virus-(infection-) associated hemophagocytic syndrome (VAHS, or IAHS)* is also frequently used (5,25), but most patients are not immuno-suppressed. Bacteria and parasites may also induce secondary HLH (5,6), as well as rheumatoid disorders. sHLH may also develop during malignancies *(malignancy-associated hemophagocytic syndrome, MAHS)*, in association with metabolic disorders, and following prolonged intravenous nutrition (fat overload syndrome) (5,6). Importantly, although sHLH may subside spontaneously, it may also be associated with pronounced mortality (5). It is important to remember that some patients with no evidence of mutations or familial disease might be affected of other presently unknown genetic defects. In these patients evidence of persistently impaired NK activity should be considered as a relevant information with possible prognostic implication (26).

NOTA BENE 1: Importantly, although HLH traditionally, and theoretically, is separated in familial (primary) and secondary HLH, this distinction may not be possible in the initial clinical setting until improved molecular diagnosis is available. Proving an acute infection at onset does not have any major therapeutic importance, since not only sHLH but also FHL often features a triggering infectious agent (27).

Therapeutic overview

(i) FHL is an invariably fatal disease, with a median survival of < 2 months after diagnosis if untreated (2). Survival and cure includes first an initial/continuation therapy, and thereafter a successful allogeneic SCT.
(ii) Patients with secondary HLH may also need initial HLH treatment. The treatment may then have to be adapted depending upon the underlying cause of the disease.

The present treatment protocol HLH-2004 has been designed for the primary, inherited disease FHL, as well as any severe form of HLH, in patients aged <18 years.

INTRODUCTION

Aims

1) To provide and evaluate a revised *initial and continuation therapy,* with the goal to initiate and maintain an acceptable condition in order to perform a curative SCT, for patients with familial, relapsing, or severe and persistent HLH.
2) To evaluate and improve the results of *SCT with various types of donors*, and to evaluate the *prognostic importance of the state of remission* at the time of SCT.
3) To evaluate the *neurological long-term complications*, with regard to early neurological alterations and CSF-findings.
4) Improved understanding of the pathophysiology in HLH by *biological studies* on genetics and cytotoxicity in affected patients, including genotype-phenotype studies and the prognostic value of NK-cell-activity subtyping.

Rationale

Ad 1. The prognosis for HLH-children has improved recently with the HLH-94 protocol (28,29). However, although HLH-94 was successful in the vast majority of the affected children that were admitted to SCT, around 20-25% of the children died during the pre-SCT phase (29). An attempt to improve the protocol is suggested. Since most of these deaths occurred during the first 2 months, this period is studied in more detail.
Ad 2. HLH-94 data suggest that although children who responded well to initial pre-SCT-induction fared best, active HLH may not automatically preclude performing SCT (30). It is important to clarify the prognostic importance of the disease activity at the time of SCT.
Ad 3. Neurological complications are the most important long-term sequelae in HLH.
Ad 4. Genotype and NK-cell-activity subtyping appear to have prognostic value (15), and patients with NK-type-3-deficiency appear to most likely require SCT to survive (22, 23).

Hypotheses

- The outcome of children with HLH may be further improved, as compared to HLH-94, by moderate modifications in the treatment protocol.
- Genotype and NK-cell-activity-subtype may have prognostic value.

SUMMARY OF THE HLH-94 RESULTS (29)

113 eligible patients aged ≤15 years from 21 countries started HLH-94 between July 1, 1994 and June 30, 1998. They all either had an affected sibling (n=25) and/or fulfilled the Histiocyte Society diagnostic guidelines (6). At a median follow-up of 3.1 years, the estimated 3-year probability of survival overall was 55% (95% confidence interval +/-9%) and in the familial cases 51% (+/-20%). Twenty enrolled children were alive and off-therapy for >12 months without SCT. For patients who were transplanted (n=65), died prior to SCT (n=25) or were still on therapy (n=3), the 3-year survival was 45% (+/-10%). The initial and continuation therapy was successful in altogether 88/113 (78%) children, in that they were either admitted for SCT (n=65) or were still alive at last follow-up (n=23). Similarly, 80% (20/25) of the patients with a positive family history received SCT. The 3-year probability of survival after SCT was 62% (+/-12%).

Survival as reported in the three largest reports on HLH.

Publication	Year	No. pts	Survival
Janka (review) (2)	1983	121	5 % (1-yr)§
Arico et al (3)	1996	122	22 % (5-yr)*
HLH-94 (29)	2002	113	55 % (5-yr)*
HLH-94 (familial cases) (29)	2002	25	51 % (5-yr)*

§ Out of 121 patients reviewed, 5/101 with follow-up data survived more than 12 months
* Probability of survival according to Kaplan-Meier estimate

DIAGNOSIS AND CLINICAL PRESENTATION

The most typical findings of HLH are fever, hepatosplenomegaly and cytopenia. Other common findings include hypertriglyceridemia, coagulopathy with hypofibrinogemia, liver dysfunction, elevated levels of ferritin and serum transaminases, and neurological symptoms that may be associated with a spinal fluid hyperproteinemia and a moderate pleocytosis (2-4, 6, 31). Other clinical findings may be lymphadenopathy, skin rash, jaundice and edema. Spontaneous partial remissions are common (32).

Histopathological findings include a widespread accumulation of lymphocytes and mature macrophages, sometimes with hemophagocytosis affecting especially the spleen, lymph nodes (if enlarged), the bone marrow, the liver and the CSF. In the liver, a histological picture similar to chronic persistent hepatitis is commonly found (33). Other frequent abnormal laboratory findings in HLH are low natural killer (NK) cell activity (12,16-22), and a hypercytokinemia in serum and the CSF (34-41), in particular elevated soluble interleukin-2 receptor (sIL-2R) levels (sCD25) (21,35,41).

Many conditions can lead to the clinical picture of HLH, including malignancies (leukemia, lymphoma, other solid tumors), infections (viral, bacterial or parasitic), and rheumatoid disorders (for MAS-therapy, see page 23). In addition, there are diseases which may resemble HLH at first look, as Langerhans cell histiocytosis, X-linked lympho-proliferative syndrome (XLP), and Chédiak-Higashi and Griscelli syndromes (5,6,42-48). Notably, XLP, Chédiak-Higashi syndrome and Griscelli syndromes have been successfully treated with the HLH-94 protocol. Other differential diagnoses are lysinuric protein intolerance (49), SCID (50), DiGeorge with HLH, and Omenn's syndrome (51).

In particular, difficulty exists in the differential diagnosis between primary and secondary HLH in non-familial cases. Viral infections, especially EBV, may trigger primary as well as secondary HLH (5). These patients may develop a severe, persistent non-familial HLH that can be treated with this protocol (52).

Acute infections may trigger FHL and cause secondary HLH, and evidence of an associated infection may therefore not have any major therapeutic importance (27). In less severe sHLH cases, either no treatment or a short duration of therapy might suffice, but future studies are necessary to define these subsets, possibly with additional genetic markers. If the disease is familial, relapsing, or severe and persistent even without family history, SCT from the best available donor is strongly recommended (29, 30).

Molecular diagnosis

In 1999, perforin gene (10q21) mutations were revealed in FHL patients (9). Later analyses revealed that they affect 20-40% of FHL patients (10). Perforin, which is co-localized with granzyme B in granule in cytotoxic cells, is secreted from cytotoxic T lymphocytes and NK cells upon conjugation between effector and target cell. In the presence of calcium it is able to insert (perforate) into the membrane of the target cell, where it polymerizes to form a cell death-inducing pore (53-55). Pore formation is suggested to lead to destruction of target cells by osmotic lysis and by allowing entrance to granzymes, which trigger apoptosis (56, 57), but perforin concentrations lower than the level necessary for pore formation together with granzyme B may induce cell death. Recent studies suggest that entry of granzyme B into target cells also can occur in a perforin-independent manner (58), but granzyme alone is not sufficient to induce toxicity.

In 2003, it was shown that mutations in hMunc 13-4 (17q25) cause FHL (23). HMunc 13-4 is essential for the priming step of cytolytic granules secretion preceding vesicle membrane fusion and a deficiency results in defective cytolytic granule exocytosis.

In XLP, 60-70% of patients have mutations in the gene SAP (SLAM-associated protein), also termed SH2-DIA (SH2-domain containing gene 1A) or DSHP. This gene, located to Xq25, regulates a protein involved in signal transduction in T and NK cells. In T cells, the protein binds to Signaling Lymphocyte Activation Molecule (SLAM, known as CDw150) and in NK cells it binds to 2B4, an NK-cell-activating receptor (43-46).

Chédiak-Higashi is linked to the LYST-gene (lyzosomal trafficking regulator gene, 1q42), Griscelli syndrome to two genes on 15q21, RAB27a (which is a key effector of cytotoxic granule exocytosis) and MYO5a (involved in organelle transport machinery) (47,48).

Clinical Diagnostic Guidelines

For many patients, molecular diagnosis is not available. Diagnostic Guidelines for HLH were presented 1991 by the FHL Study Group of the Histiocyte Society, see below (6), based on common clinical, laboratory and histopathological findings.

The 1991 Diagnostic Guidelines for HLH* (adapted from ref 6)

Clinical criteria
* Fever
* Splenomegaly

Laboratory criteria
* Cytopenias (affecting ≥ 2 of 3 lineages in the peripheral blood:
  Hemoglobin (< 90 g/L), Platelets (<100 x 10^9/L), Neutrophils (<1.0 x 10^9/L)
* Hypertriglyceridemia and/or hypofibrinogenemia
  (fasting triglycerides ≥2.0 mmol/L or ≥3 SD of the normal value for age,
  fibrinogen ≤1.5 g/L or 3 SD)

Histopathologic criteria
* Hemophagocytosis in bone marrow or spleen or lymph nodes.
  No evidence of malignancy

Revision of Diagnostic Guidelines for HLH-2004

As mentioned already in the 1991 publication on Diagnostic Guidelines, HLH "may also have an atypical and insidious course in some patients, in whom all criteria not always are fulfilled" (6). Moreover, a number of patients may develop one or more of the diagnostic criteria late during the course of the disease (6, 59).

Based on these findings, and the added knowledge on molecular diagnosis, the diagnostic guidelines have been revised. First, patients with a molecular diagnosis of primary HLH do not need to also fulfill the diagnostic criteria.

Second, additional criteria are introduced:
A. Low or absent NK-cell activity (according to local laboratory reference).
B. Ferritin >500 microgram/L
C. Soluble CD25 (i.e. soluble IL-2 receptor) >2400 U/ml.

Ad NK-cell activity: NK-cell activity is well-known to most commonly be low or absent in HLH (12, 16-22). Preliminary data indicate that almost all PRF1 deficient patients have abnormal NK cell activity.

Ad Ferritin: In the HLH-94 study, 48 eligible children registered 1994-June 2002 had familial disease (defined as an affected sibling). Data on ferritin, an important diagnostic parameter (31), was available for in 31 children, 26 of whom had >500 microgram/L (sensitivity 0.84) and 23 had ferritin >1000 microgram/L (sensitivity 0.74).

Ad Soluble CD25: Soluble CD25 (>2400 U/ml) appears to be a valuable serum parameter in the diagnosis of HLH (12, 15-19). When compared with other diseases (sepsis, juvenile myelo-monocytic leukemia, Langerhans cell histiocytosis), specificity was 1.0 and sensitivity 0.93. The corresponding values for CD95 ligand (>500 pg/ml) were 1.0 and 0.72 (60). These markers are not readily available for many patients.

TABLE 2. DIAGNOSTIC GUIDELINES FOR HLH-2004 (revision of ref 6)

The diagnosis HLH can be established if one of either 1 or 2 below is fulfilled.

1. A molecular diagnosis consistent with HLH.

2. Diagnostic criteria for HLH fulfilled (5 out of the 8 criteria below).

A) Initial diagnostic criteria (to be evaluated in all patients with HLH).

Clinical criteria
* Fever
* Splenomegaly

Laboratory criteria
* Cytopenias (affecting ≥ 2 of 3 lineages in the peripheral blood:
  Hemoglobin (<90 g/L), Platelets (<100 x 10^9/L), Neutrophils (<1.0 x 10^9/L)
  (In infants <4 weeks: Hemoglobin <100 g/L)
* Hypertriglyceridemia and/or hypofibrinogenemia
  (fasting triglycerides ≥3.0 mmol/L (i e ≥265 mg/dL), fibrinogen ≤1.5 g/L)

Histopathologic criteria
* Hemophagocytosis in bone marrow or spleen or lymph nodes.
  No evidence of malignancy

B) New diagnostic criteria.

* Low or absent NK-cell activity (according to local laboratory reference)
* Ferritin ≥500 microgram/L
* Soluble CD25 (i.e. soluble IL-2 receptor) ≥2400 U/ml

Comments:
1. If hemophagocytic activity is not proven at the time of presentation, further search for hemophagocytic activity is encouraged. If the bone marrow specimen is not conclusive, material may be obtained from other organs. Serial marrow aspirates over time may also be helpful.

2. The following findings may provide strong supportive evidence for the diagnosis:
(a) Spinal fluid pleocytosis (mononuclear cells) and/or elevated spinal fluid protein,
(b) Histological picture in the liver resembling chronic persistent hepatitis (biopsy).

3. Other abnormal clinical and laboratory findings consistent with the diagnosis are:
Cerebromeningeal symptoms, lymph node enlargement, jaundice, edema, skin rash.
Hepatic enzyme abnormalities, hypoproteinemia, hyponatremia, VLDL ↑, HDL ↓.

NOTA BENE 2: Not all patients do fulfil all the diagnostic criteria presented in Table 2. Moreover, a number of patients may develop one or more of the diagnostic criteria late during the course of the disease (6,59). Thus, **therapy may sometimes have to be commenced on strong clinical suspicion of HLH**, before overwhelming disease activity makes irreversible damage and a response to treatment less likely. (Contact your local subcenter or local coordinator in case of questions).

NOTA BENE 3: There are no reliable criteria to distinguish primary and secondary HLH, clinically and histologically. The onset of FHL is most common in infancy, but has been reported also in adolescents and young adults (61,62). Secondary HLH is found in all ages. In infants, a primary cause of HLH is more likely than a secondary cause.

THERAPEUTIC BACKGROUND

Chemotherapy: Without treatment, FHL is usually rapidly fatal and a median survival of two months has been reported (2,24). A number of treatments including cytotoxic agents were initially tried with no or moderate effect (2). Repeated plasma or blood exchange induced transient resolution in some patients (63). The use of the epipodophyllotoxin derivatives etoposide (64) and later teniposide (65) in combination with steroids were both shown to induce prolonged resolution. A treatment protocol including etoposide, steroids, intrathecal methotrexate and cranial irradiation was shown to be successful in inducing resolution and prolonged survival (66). Later, a therapeutic regimen that also included guidelines for the maintenance therapy and reactivation was presented, based on similar drugs but the cranial irradiation had now been excluded (32). This treatment has been effective in prolongation of survival, in some patients >5 years after onset, but it has not been possible to ultimately cure any child with familial disease with chemotherapy alone (29).

The biology of the remarkably beneficial effects of etoposide in HLH, previously not well understood, may be explained by the recent findings that FHL is associated with a defective triggering of apoptosis, and that etoposide is known to be an excellent initiator of apoptosis (8, 67). Similarly, the effect of dexamethasone might be explained by its anti-inflammatory and pro-apoptotic properties, particularly valuable since the drug also penetrates well into the CNS, and CSA is known to reduce T-cell activity, which is increased in HLH. An epipodophyllotoxin derivative (etoposide) and corticosteroids (dexamethasone) were used in the HLH-94 protocol (29).

SCT: A major therapeutic breakthrough was achieved when allogeneic hematopoietic SCT was shown to induce not only a prolonged resolution but also cure (68). Allogeneic SCT is necessary to cure a child with FHL (68-72). Recent SCT series have reported data ranging from a 3-year probability of survival of 45 % (n=20) to an overall survival of 64% with HLA-nonidentical donors (n=14) and 100% in a single-center material with matched sibling donors and unrelated donors (n=12) (73-76).

CNS disease: Cerebral involvement may cause severe and irreversible damage (59,77-80) and intrathecal therapy has been used although its therapeutic effect neither has been sufficient nor persistent. In children with HLH CNS disease at diagnosis often resolve with systemic therapy whereas intrathecal therapy appear less effective. Therefore, systemic therapy including dexamethasone, which penetrates the blood-brain barrier well, was first line therapy in HLH-94, also in case of CNS involvement. Intrathecal therapy may be added in certain clinical situations, see pages 18 and 22.

Immunotherapy: The immunosuppressive drug cyclosporin A (CSA) has shown to be effective in FHL (80-82). Also, ATG has been successful in inducing resolution (82). Still, a majority of the patients who were not transplanted in the months following ATG treatment, relapsed in the CNS despite CSA therapy. In HLH-94, CSA was *combined* with steroids and VP-16 (28, 29).

Virus-infections associated with onset of the disease: FHL is often triggered by an infection. Thus, the presence of a virus-infection, such as EBV, in a child with HLH does not rule out an inherited disease, i.e. FHL (27). In addition, clinical features of numerous EBV-associated cases are controlled only by continuous administration of chemotherapy (52, 83). The prognosis for children with HLH is poor whether a virus-infection is associated or not (6,27,84). Therefore, in HLH-94 all children with HLH were initially started on chemotherapy, whether a virus-infection was associated with the onset or not.

HLH-94: In HLH-94, the initial treatment was based on etoposide (initially twice weekly, then once weekly) and corticosteroids (in line with a previously presented regimen, 32) followed by a continuation therapy with etoposide and steroid pulses, in combination with CSA and, in selected cases, intrathecal methotrexate. In addition, SCT was suggested for children with persistent and reactivating disease (28, 29).

CONCLUSIONS FROM HLH-94

The survival results with the HLH-94 protocol exceeded our expectations (29). More children than expected survived the intensive disease phase by using the initial and continuation therapy, and hence more children could to be admitted to SCT. Moreover, more patients than expected survived the allogeneic SCT.

Overview of the outcome in HLH-94 during the first 4 years (from ref 29)

In children with an affected sibling, i.e., verified familial disease, the 3-year probability of survival (pSU) was 51% (95% confidence level ±9%) for eligible patients recruited during the 4-year period July 1994 - June 1998. (Eligibility defined as no previous cytotoxic or CSA treatment, familial disease or all diagnostic criteria fulfilled, and HLH-94 therapy commenced prior to July 1, 1998).

At a median follow-up of 3.1 years, the estimated 3-year probability of survival overall was 55% (+/-9%) (n=113). Twenty enrolled children were alive and off-therapy for >12 months without SCT. For patients who were transplanted (n=65), dead prior to SCT (n=25) or were still on therapy (n=3), the 3-year survival was 45% (+/-10%). The 3-year probability of survival after SCT was 62% (+/-12%).

In brief, 25 of the eligible 113 patients (22%) died prior to SCT (for details see below). In addition, 25 children died after SCT. The present protocol is aiming to improve the results further.

Initial treatment (week 1-8)

Not surprisingly in a disease characterized by severe cytopenia and an immune deficiency, dose modifications in HLH-94 were common. In particular, the treatment of VP-16 was decreased in a substantial number of the patients. For dexamethasone, the amount administered was increased in more patients than it was decreased.

During the first 4-years of analysis, 6 patients died during the first month of treatment and 6 additional during the second month of treatment. It is suggested to increase treatment intensity during the first 2 months of therapy, with a drug that does not induce increased myelotoxicity.

Proposed action:
- CSA, previously introduced after 8 weeks, is instead initiated at onset.

Neutropenia at onset of the Initial treatment (week 1)

In our opinion, neutropenia at onset is caused by the disease, and it does therefore not in itself justify dose reduction. Proposed action if ANC at onset of treatment is $<0.5 \times 10^9$/L and the bone marrow is hypocellular (which is only rarely the case): Consider to omit the first two doses of VP-16, and to discuss the treatment with the local sub-center.

Neutropenia developing during the Initial treatment (week 2-8)

- If the disease has started to regress (fever subsides, platelet count improves), one or two doses of etoposide may be omitted if the bone marrow is hypocellular, during which period dexamethasone is administered at 10 mg/m2, and CSA as scheduled. Consider to discuss the treatment with the local sub-center.

- If the disease has not at all started to regress: This is a very difficult situation, that is recommended to be discussed with the local sub-center. Consider the possibility of an ongoing (viral) infection triggering the immune system, and appropriate therapy.

Continuation therapy (week 9-)

Of the six children who died during weeks 9-24 of the HLH-94 protocol, all were reported as dead of disease, at least three of whom had CNS-involvement.

Proposed action:

- Since CNS reactivations may occur during continuation therapy, it is suggested to analyse CSF (at least for cells and protein, and cytospin if CSF-pleocytosis) if there is a systemic reactivation or neurological symptoms, in order to detect reactivation in the CNS early. Additional information may be provided by cytokine analysis (as neopterin) (37). Brain MRI is also highly recommended.

- In case of reactivation during the continuation therapy, it is recommended to restart at week 2 of the protocol, see separate paragraph (page 22). In this case, the initial therapy period may be shorter, and the continuation therapy may be more intensive, and continuous dexamethasone 2.5 mg/ m^2 between the dexamethasone pulses may be considered.

- In addition, if the patient is a candidate for SCT it should be performed as soon as an acceptable donor is available. If no other donor is available, SCT with a haplo-identical family donor is suggested, to be performed at an experienced SCT center (see SCT chapter, below and page 25).

Intrathecal therapy

With available HLH-94 data, it has not yet been possible to determine whether intrathecal therapy is beneficial or not, in addition to systemic HLH-94 therapy (29). It is the opinion of the Study Committee that systemic therapy, in particular with corticosteroids, will reduce CNS disease activity, in particular CNS activity at diagnosis. It can not be ruled out that intrathecal therapy may have additional beneficial effects, at least in some patients. Intrathecal therapy may be beneficial in patients with CNS reactivation, and is suggested in case of CNS reactivation.

As in HLH-94, up to four intrathecal doses are recommended week 3, 4, 5 and 6, but only if the neurological symptoms are progressive during the first two weeks, or if an abnormal CSF at onset has not improved after two weeks. Having the beneficial effect of systemic corticosteroids in mind, it is suggested to add corticosteroids to the IT MTX when IT therapy is administered to the patients with CNS involvement.

Stem cell transplantation (SCT) (see also page 25)

Analysis of SCTs performed 1995-2000 revealed an overall estimated 3-yr-survival post-SCT of 64% (+/-10%) (n=86); 71% (+/-18%) with matched related donors (MRD, n=24), 70% (+/-16%) with matched unrelated donors (MUD, n=33), 50% (+/-24%) with family haploidentical donors (n=16), and 54+/-27% with mismatched unrelated donors (n=13) (78). Univariate analysis (n=86) revealed a lower 3-yr-survival in children with active disease at SCT (54%, n=37) as compared to children with non-active disease (71%, n=49) (p=0.065). There was a non-significant trend towards better survival in children that had received etoposide as part of their conditioning (70% versus 58%, univariate analysis). In summary: 1/ the cure rate with HSCT using MRD or MUD is not markedly different, and acceptable also with mismatched donors (considering that SCT is necessary for cure in FHL), 2/ active disease should probably not automatically preclude performing SCT, and 3/ inclusion of etoposide in the SCT-conditioning may improve survival further.

GENERAL STUDY DESIGN

For general overview, see Figure 1. The HLH-2004 protocol is designed for the primary, inherited disease FHL, but may be beneficial in patients with secondary HLH as well. The protocol is based on etoposide, steroids, cyclosporin A, intrathecal therapy in selected patients (methotrexate and prednisolone), and SCT. The major aim is to achieve a clinically stable resolution of the disease and to cure by SCT.

Following 8 weeks of initial therapy, all children with familial disease or with a diagnosis verified by molecular biology, as well as children with a non-familial disease that is severe and persistent, or reactivated, continue with VP-16/steroids in combination with cyclosporin A immunotherapy. SCT is performed as early as possible, when an accepted donor is available. In non-familial cases, treatment is stopped in patients with a complete resolution of disease after 8 weeks of initial therapy, in order to avoid SCT in a child with an HLH which may be an unrevealed secondary disease.

In children with secondary HLH, such as infection-associated HLH or malignancy-associated HLH, the underlying cause of the immune activation is treated first. If necessary, chemo-immunotherapy is also administered, as in HLH-2004.

Declaration of intent

In many children it may not be possible to determine whether the disease is a primary, inherited disease or a secondary HLH. If the disease is severe and persistent, or reactivating, treatment according to HLH-2004 is suggested, initially for 8 weeks. Be aware that a viral infection, such as EBV and CMV, may trigger a primary HLH.

The intention with this protocol is that children with primary HLH will receive continuation therapy and SCT. In children with secondary HLH, first the cause of the immune activation is treated and, if necessary, HLH-2004 is also administered. If it is unknown whether the disease is primary or secondary and a thorough investigation has revealed no underlying malignancy, no bacterial or parasitic infection and no other cause of the immune-activation, the patient is administered initial therapy, whether a viral infection is associated or not. Treatment is stopped after 8 weeks, if the disease has had a complete resolution. If the disease is severe and persistent, or reactivating, continuation therapy and SCT is suggested.

Brief protocol overview (see also Figure 1-2)

Ad Initial therapy: At diagnosis many of the patients are critically ill and the major aims of the initial therapy are 1/ to keep the patients alive and to reduce the number and degree of permanent complications during this critical period and 2/ to achieve a resolution of the disease. The initial therapy covers the first 8 weeks of treatment and includes VP-16, dexamethasone, CSA, and, in selected patients, intrathecal therapy (methotrexate and prednisolone) (see page 22).

Ad Continuation therapy: The major aim is to sustain the resolution of the disease. SCT is performed when an accepted donor is available. The therapy is intensive with a combination of VP-16, dexamethasone pulses and cyclosporin A in order to reduce the risk of reactivation. Since the disease activity is different in each child, the therapy may have to be further intensified in some patients, (see page 22).

Ad SCT: SCT is recommended as soon as an accepted donor is available and is preferably made when the disease is in resolution. However, active HLH disease should probably not automatically preclude performing SCT (page 25) (30). The choice of performing SCT or not, as well as the choice of donor, is made by the treating physician.

PATIENT'S ELIGIBILITY

All newly diagnosed patients who meet the following criteria are eligible to be fully enrolled and followed in the study:
- Patients who fulfil the diagnostic criteria of HLH.
- Age < 18 years at onset of therapy.
- No prior cytotoxic or cyclosporin A treatment for HLH.

Patients with HLH starting the HLH-2004 protocol who do not fulfil the diagnostic criteria or aged ≥18 yrs may also be registered in the study but will be studied separately.

Patients with XLP, Chediak-Higashi syndrome, Griscelli syndrome, and similar syndromes, as well as patients with macrophage activation syndrome (MAS) secondary to known rheumatoid diseases may also be registered, and will be studied separately.

It must be emphasized that patients with active HLH may be extremely sick but that this is no reason to avoid treatment since the initial therapy commonly induces a rapid regression of the symptoms.

Any doctor or patient is free to leave the study at any time. A sample for informed consent is provided in the Appendix.

PRE-TREATMENT INVESTIGATIONS

Clinical

* Complete history:
Family history (consanguinity, previous childhood deaths in this family or relatives, late miscarriages of the mother), recent infections and vaccinations, previous bouts with similar symptoms, fever (duration and level), neurological symptoms (including irritability, ataxia, convulsions and others), edema, jaundice, skin rash.

* Complete physical examination:
Temperature, height, weight, skin rashes, jaundice, purpura, bleeding, edema, tonsillitis, lymphadenopathies, dyspnea, tachypnea, liver size, spleen size, ascites, blood pressure, neurological examination incl cranial nerve abnormalities and cerebellar dysfunction.

Laboratory and Radiographic

Baseline evaluations for all patients:
- Hemoglobin, WBC and differential, platelet count, reticulocytes, ferritin
- Liver function (serum transaminases, bilirubin, albumin, LDH)
- Coagulation profile (fibrinogen, APTT, PT, D-dimers)
- Lipid evaluation (fasting triglycerides)
- Kidney function (creatinine) and serum electrolytes
- Soluble IL-2 receptor (sCD25) is not readily available, but suggested if available.
- Immunoglobulin levels (including also IgA)
- Spinal tap
    - cell and protein content (consider to add lactate and glucose)
    - morphological and immunological analyses (if cells in CSF)

- Infectious investigation including CMV, EBV, HIV, HSV, HHV6+8, rubella, varicellae virus, parvovirus, adenovirus and other appropriate viruses. It is suggested that the investigations include PCR. Consider the diagnoses leishmaniasis, brucellosis, tuberculosis, mycoplasma, syphilis, among others.

- Bone marrow aspiration (hemophagocytosis, differential diagnosis evaluation) (consider a bone marrow biopsy in case of dry tap or a diluted marrow sample)
- Fine needle aspiration biopsy of an enlarged lymph node or a liver biopsy may also be valuable, (see also Diagnostic Guidelines, page 15).

- NK-cell activity (studied at specific study laboratories, see page Appendix-19).

- Molecular diagnosis (perforin, hMunc 13-4 and relevant other genes).
  Some institutions perform flow cytometry screening for perforin in NK cells and cytotoxic T cells (12). For addresses to study laboratories, see page Appendix-19.

- Glomerular filtration rate (because of cyclosporin therapy), as soon as feasible, at least in patients with elevated creatinine levels.

- Imaging
  - Abdominal ultrasound (or CT) (liver & spleen size, other abnormalities)
  - Chest X-ray (or CT of the chest) (pulmonary infiltrates)
  - MRI of the brain is recommended, since CNS affection is common, and it is strongly recommended in patients with neurological symptoms

- HLA-typing of the patient and the family is made as soon as feasible. We recommend a preliminary donor search in infants (< 6 mos at diagnosis) even if the patient has a full response and is eligible to stop therapy at 8 weeks.

MONITORING

- For monitoring and assessment, see Table I (page 10).

- Follow-up evaluation for the study are made after 2 month, 6 months, 12 months and later on once yearly. If SCT is made, change to SCT +100, SCT +1yr, and thereafter yearly, see follow-up report forms in the Appendix.

- The documentations sheets for the initial and the continuation therapy, as well as the follow-up sheets, are sent to the local sub-center.

TREATMENT

ACUTE MANAGEMENT

The initial therapy covers the initial 8 weeks of treatment. It includes etoposide, dexamethasone, CSA, and, in some patients, intrathecal therapy. **The dosages are calculated per m^2 also in children less than 10kg.**

Early supportive therapy:
* Maximal supportive care.
* Appropriate broad-spectrum antibiotics (until culture results are available).

Further and continuous recommended supportive therapy:
* Prophylactic cotrimoxazole, eq 5 mg/kg of trimethoprim, 2-3 times weekly.
* An oral antimycotic, at the choice of the physician, during the initial therapy.
* Consider antiviral therapy in patients with ongoing viral infections.
* IvIG (0.5 g/kg iv) once every 4 weeks (during the initial and continuation therapy).

INITIAL THERAPY

1. Etoposide
- 150 mg/m^2 iv twice weekly (week 1-2). Only in certain conditions, if ANC <0.5 x10^9/L and the bone marrow is hypocellular (which only rarely is the case), can these be omitted.
- 150 mg/m^2 iv once weekly (week 3-8).

2. Dexamethasone
- Dexamethasone 10 mg/m^2 daily, for the first 2 weeks (week 1-2).
- Dexamethasone 5 mg/m^2 daily, for another 2 weeks (week 3-4).
- Dexamethasone 2.5 mg/m^2 daily, for another 2 weeks (week 5-6)
- Dexamethasone 1.25 mg/m^2 daily, for another week (week 7).
  Steroids are tapered and discontinued during week 8.
* Gastroprotection with ranitidine or other gastroprotective agent is suggested.

3. Cyclosporin A
- The blood levels determine the dosages, aim at levels around 200 microgram/L (trough value) (monoclonal antibody assay of whole blood). Start with 6 mg/kg daily (divided in 2 daily doses) already week 1, if kidney function is normal.

4. Intrathecal injections with methotrexate and prednisolone
The CSF is evaluated at diagnosis and after 2 weeks. If after 2 weeks there is clinical evidence of progressive neurological symptoms or if an abnormal CSF (cell count and protein) has not improved, additional CNS-therapy is initiated with 4 weekly intrathecal injections. Be aware that some patients may have increased intracranial pressure.
- Methotrexate: <1 yr 6 mg, 1-2 yrs 8 mg, 2-3 yrs 10 mg, >3 yrs 12 mg.
- Prednisolone: <1 yr 4 mg, 1-2 yrs 6 mg, 2-3 yrs 8 mg, >3 yrs 10 mg.

CONTINUATION THERAPY

The continuation therapy is a continuation of the initial therapy with the major aim to keep the disease non-active week 9-40. Increasing disease activity may make it necessary to intensify the treatment in some children. Patients with non-familial disease and no genetic evidence of HLH, are suggested to start continuation therapy only if the disease is active after the initial therapy (see also flow-sheet in Fig 1, page 5).

1. Etoposide
- 150 mg/m^2 iv, every second week.

2. Prednisolone
- Dexamethasone pulses every second week, 10 mg/m^2 for 3 days.

3. Cyclosporin A
- Aim for blood levels around 200 microgram/L, as above. Monitor GFR.

SUBSEQUENT THERAPY (in non-SCT patients)

In children with primary HLH, cure can be achieved only by SCT. Even in perforin deficient patients transient treatment-induced or even spontaneous resolution may be observed, but ultimately all these patients will end up in progressive disease. If a matched donor can not be found, mis-matched donor SCT is suggested, as with a family haploidentical donor. The aim of the subsequent therapy is to sustain a resolution in patients where SCT has not been performed, if possible with a reduction of therapy. In secondary HLH, treatment should not continue beyond 40 weeks, usually only 8 weeks are necessary. Four treatment strategies are offered, and treatment can be tapered and stopped if there is no reactivation. The treating physician may choose either one:

1. Continue the continuation therapy as it is (as week 9-40).
2. Prolong the intervals between each VP-16 infusion and dexamethasone pulse from 2 to 4 weeks, and continue CSA as previously. Thus, the patient will receive alternating treatment every second week (instead of weekly) with VP-16 or dexamethasone pulse.
3. Exclude VP-16. Continue with CSA and dexa only, in doses and interval as week 9-40.
4. Exclude VP-16. Continue with CSA or dexa only.

It must be noted that many patients may have to go back to the initial continuation schedule, since a reduced treatment will not be enough to keep the disease non-active.

REACTIVATION THERAPY

Reactivations may occur following immune response triggering, such as infections and vaccinations. In case of reactivation, consider broad-spectrum antibiotics, antiviral therapy, and antifungal therapy.

FHL is characterized by frequent reactivations, or even a more or less continuous disease activity. In particular, reactivation of the disease is common as the therapeutic intensity is reduced. Accordingly, a reactivation will commonly respond to an intensification of the ordinary therapy. Treatment of a reactivation has to be individualized for each patient.

Suggested action if the patient develops a reactivation:
1. It is recommended to intensify therapy, such as to restart from wk 2, but the initial therapy may be less than 8 wks, and then continue with modified continuation therapy.
2. Add intrathecal therapy in case of CNS-reactivation.
3. Consider dexamethasone daily, also between the dexa-pulses, in continuation therapy, but be aware that it may lead to severe side-effects, so an early SCT is then suggested.
4. If inadequate response, contact your local sub-center.

MACROPHAGE ACTIVATION SYNDROME

Macrophage activation syndrome (MAS), a serious complication of rheumatoid arthritis and other childhood systemic inflammatory disorders, is thought to be caused by excessive activation and proliferation of T lymphocytes and macrophages. The recognition that MAS belongs to the secondary or reactive hemophagocytic syndromes has led to the proposal to rename it according to the contemporary classification of histiocytic disorders (85, 86). In addition to corticosteroids, CSA has been found effective in patients with corticosteroid-resistant MAS (87).

SALVAGE THERAPY

The current protocol does not include a salvage protocol. We want to mention an alternative approach of inducing remission, with a regimen including a treatment with steroids (2 to 5 mg/kg/d methylprednisolone intravenously, followed by progressive tapering) and ATG (82). There have only been a small fraction of the patients that did not respond to some degree to the HLH-94 protocol, and many of these did not respond to ATG either. It is therefore suggested to discuss salvage therapy with the local sub-center. In case of reactivation after graft rejection, it is suggested to restart from wk 2. Note that early after SCT the immunosuppression may induce a secondary HLH picture, which may be due to late lymphocyte recovery, necessitating HLH therapy.

In brief: It is recommended to discuss non-responders with the local study coordinator.

ENDING THERAPY

Ending therapy is only recommended in children with resolution of the disease. Close follow-up including signs of reactivation are warranted (such as fever, hepato-splenomegaly, neurological abnormalities; hemoglobin, platelets, WBC, ANC, ferritin, transaminases).

DEFINITION OF DISEASE STATES

*** Clinical response:**

Criteria to be used during the induction therapy (at 2 weeks and 4 weeks) on whether to continue the therapy as out-lined:

- No fever
- Reduction of spleen size
- Platelets $\geq 100 \times 10^9$/L
- Normal fibrinogen
- Decreasing ferritin levels (by 25%)

If not all criteria are fulfilled, contact your local sub-center to discuss further therapy.

*** Non-active disease (resolution):**

Criteria to be used at the decision-point on whether to continue therapy after 8 weeks:

- No fever
- No splenomegaly (isolated moderate splenomegaly may persist in some patients)
- No cytopenia (Hb ≥90 g/L, platelets $\geq 100 \times 10^9$/L, ANC $\geq 0.5 \times 10^9$/L)
- No hypertriglyceridemia (<3mmol/L, i.e. <265 mg/dL)
- No hyperferritinemia ≥500 μg/L
- Normal CSF (for previously CSF positive patients)
- (Decrease of sCD25 in case the test is available)

*** Active disease:**

Patients that do not have non-active disease, as defined above.

*** Reactivation of disease:**

Children that have achieved a remission, and then again develop ≥ 3 of these 8 signs:

- Fever
- Splenomegaly
- Platelets $< 100 \times 10^9$/L
- Hypertriglyceridemia (fasting level ≥3.0 mmol/L, i.e. ≥265 mg/dL)
- Hypofibrinogenemia ≤1.5 g/L
- Hemophagocytosis
- Increasing ferritin levels
- Soluble CD25 (i.e. soluble IL-2 receptor) ≥2400 U/ml

The development of new CNS symptoms are sufficient as a single criterion for reactivation.

STEM CELL TRANSPLANTATION (SCT)

In primary HLH, i.e. FHL, allogeneic SCT is the only curative therapy (29, 68-76). Some major problems are 1/ to find an acceptable SCT-donor and 2/ to keep the patients alive and without sequelae until the SCT is performed.

In familial disease and in severe and persistent non-familial disease, SCT is made, preferably when the disease is non-active, when an acceptable donor is available. In non-familial disease with complete resolution after the initial 8-week therapy, SCT is performed only if the disease has been reactivated (indicating a primary disease).

An HLA-identical donor is preferable. The risk of a sibling carrying the disease must be considered and is less likely if using an older sibling, but this age criteria cannot be used as an indicator for being non-affected. If a genetic marker (as perforin/hMunc) is not available, NK-cell activity has been considered as a surrogate marker of immune dysfunction, but recent data suggest that healthy siblings may also have low NK-cell activity (20). If an HLA-identical relative is not available, SCT with a matched unrelated donor is recommended. If there is no matched donor available, a mismatched donor (including a haploidentical family donor) or cord blood is suggested, upon the decision of the physician. The results with mismatched donors are improving (73-76). At the decision of the physician, PBSCT may be considered, particularly if marrow is not available.

Outcome after SCT in HLH-94 (ref 29)

SCT donor	All cases (n=65)	Alive (%) (n=40)
Matched related donor	15	10 (67%)
Matched unrelated donor	25	17 (68%)
Mismatched unrelated donor	4	1 (25%)
Family haploidentical	14	6 (43%)
Cord	5	4 (80%)
Incomplete data*	2	2

* Includes: related donor with match not reported (n=2, both alive)

The preparative treatment for SCT and the GVHD prophylaxis is up to the treating physician and the transplantation unit; a suggestion is provided below. However, we would advise to also include etoposide, in addition to busulfan and cyclophosphamide, in the conditioning regimen, in accordance with previous experience (29-30, 73-76).

SUGGESTION FOR SCT REGIMEN

Preparative Regimen

* Day -8 Busulfan 2mg/kg po, or equivalent iv (as 1.6mg/kg), twice daily.
* Day -7 Busulfan 2mg/kg po, or equivalent iv (as 1.6mg/kg), twice daily.
* Day -6 Busulfan 2mg/kg po, or equivalent iv (as 1.6mg/kg), twice daily.
* Day -5 Busulfan 2mg/kg po, or equivalent iv (as 1.6mg/kg), twice daily.
* Day -4 Etoposide 30 mg/kg iv (6 hr infusion) (maximum 1800 mg)
* Day -3 Cyclophosphamide 60 mg/kg iv (1 hr infusion)
* Day -2 Cyclophosphamide 60 mg/kg iv (1 hr infusion)
* Day 0 Marrow infusion (preferably $\geq 3 \times 10^8$ nucleated cells/kg, non T-cell-depleted).

Graft-vs-Host Disease (GVHD) Prophylaxis

1. Cyclosporin continuous infusion starting day -1 pre-transplant with 3 mg/kg until oral nutrition re-established, thereafter 12.5 mg/kg orally daily. CSA dosage is adjusted according to monitoring of CSA through concentration levels. The immunosuppression is discontinued after 6-12 months, if possible.

2. Short course methotrexate:
* Day +1 15 mg/m^2 iv
* Day +3 10 mg/m^2 iv
* Day +6 10 mg/m^2 iv

Methotrexate may be substituted by mycophenolate mofetil (MMF) (as in cord blood transplants and in patients with decreased liver function) 15 mg/kg x 2 daily orally, starting on day 0 and given until day 40 post transplant, then tapered to be discontinued.

Additional Treatment for Unrelated Donor Transplants

* ATG (Antithymocyte Globulin) (12 hour infusion iv) on days –3, -2 and –1.
Adjust ATG dosage according to manufacturers recommendation.

(Methylprednisolone 2 mg/kg iv and clemastinhydrogenfumarate 1 mg iv 30 min prior to each ATG infusion.)

* Metronidazole 22 mg/kg daily (po or iv) from day –8 until discharge.

Supportive Care Guidelines

The supportive care is up to the treating physician and the transplant center. Suggestions:

* Monitoring of busulfan concentration
* Clonazepam or phenytoin administered before and parallel to busulfan as anticonvulsive prophylaxis
* Dexamethasone days –4, –3 to prevent VP-16 induced anaphylactic-like symptoms
* Mesna for uroprotection in association with cyclophosphamide infusions
* Hydration iv 2-3 L/m^2/24h from start of conditioning through 24 hours after last dose of cyclophosphamide
* Trimethoprim/Sulfamethoxazole: pneumocystis prophylaxis 2-3 days/week
* Acyclovir prophylaxis day +1 until +100, at least to pat with high herpes simplex titers

Pre and Post Transplant Monitoring for the Study

* Disease activity status prior to SCT conditioning; at day +100, at 1-year, at 2-year, etc
* CNS disease activity within 2 weeks prior to SCT (raised cell count or high protein)
* MRI of the CNS prior to SCT conditioning, and at +1-year if abnormal pre-SCT
* NK and CTL-cell activity prior to SCT conditioning, and at day+100 and at +1-year
* Lansky play scale: at SCT, and at day+100, +1-year, etc (see Appendix for definition)

* Engraftment (first day with ANC >0.5 x 10^9/L)
* Chimerism analysis day +100, and at 1-year recommended.
* Staging for acute GVHD (ref 88)
* Staging for chronic GVHD (ref 89)

Additional Pre and Post Transplant Monitoring
* Hepatic veno-occlusive disease (VOD):
  Modified Seattle criteria (ref 90). Bilirubin ≥34.2 μ/L (before day 20) + weight gain ≥5%, liver ≥3cm more than baseline under the costal margin.
* Mucositis staging (ref 91)
* Capillary leak syndrome defined as:
  Generalized edema + weight gain ≥10% + pleural effusions or ascites

  (There is a potential risk for hypercytokinemia with engraftment +/- acute GVHD – an emergency that could be treated with very high doses of steroids).

Reduced intensity conditioning

There is as yet limited data available on reduced intensity conditioning in HLH. It is not possible at this time to make any firmly based suggestions on such regimens in HLH.

DRUG INFORMATION AND TOXICITY

Etoposide

Dilute to a concentration of not more than 0.4 mg/ml in 0.9% sodium chloride or 5% dextrose. Administer as intravenous infusion over 1-3 hours.

Toxicity: Myelosuppression (leukopenia, thrombocytopenia), hypotension (if the drug is infused too rapidly), hepatocellular damage, nausea, vomiting, fever, headache, abdominal pain, diarrhoea, anorexia, alopecia, allergic reactions, and, rarely, second malignancies (leukemia/myelodysplastic syndrome). Second malignancies are very rare in HLH (three cases reported and one unpublished) (29,92,93). Etoposide is included in the protocol since it has shown to have such a positive effect in FHL, which without treatment is uniformly fatal. Etoposide has also been used in familial cases diagnosed and treated with chemotherapy in utero (94).

Dexamethasone

Increased appetite, centripetal obesity, fluid retention, hyperglycemia, immunosuppression, myopathy, osteoporosis, aseptic necrosis, peptic ulceration, pancreatitis, mental alteration, cataracts, hypertension, precipitation of diabetes, growth failure, amenorrhea, impaired wound healing, atrophy of subcutaneous tissue.

Cyclosporin A

Nephrotoxicity (usually dose dependent but may be irreversible), hypertension, hypertrichosis, nausea, vomiting, anorexia, gingival hyperplasia, liver affection with elevation of serum transaminases and bilirubin, tremor, hypomagnesemia, tiredness, paresthesia, edema, headache, convulsions (less common), diarrhoeas, rash, weight gain, elevation of serum potassium and uric acid, and anaphylactic reactions (rare).

THERAPY MODIFICATIONS

If <10kg: The dosages are calculated per m^2 also in children less than 10kg.

HLH may cause a wide variety of symptoms and it may be difficult to evaluate whether a sign or symptom is due to the therapy or due to the disease. The treatment may have to be individualized.

Bone marrow toxicity: Cytopenia is a common sign of disease activity, particularly at onset, but myelotoxicity may occur following intensive etoposide treatment as well. Delayed administration or reduced doses may be necessary, in particular if no other signs of disease activity are available (see page 24). The differential diagnosis between cytopenia induced by the disease or by etoposide myelotoxicity may be difficult. High serum ferritin, persistent thrombocytopenia, or a persistently elevated sCD25 strongly suggests persistence of basal disease rather than therapy-induced myelotoxicity. Consider bone marrow examination.

Nephrotoxicity: Cyclosporin A may cause irreversible kidney damage. In case of increasing creatinine and urea values or other signs of decreasing kidney function, a reduction of the cyclosporin dosage has urgently to be considered. Check GFR.

Hepatotoxicity: The disease itself may markedly affect liver function and a dose reduction due to the therapy is rarely necessary.

Neurotoxicity: Intrathecal methotrexate may cause neurotoxicity. Cyclosporin has been related to neurological complications. However, many patients with HLH also develop CNS-disease and this has to be urgently considered.

DATA COLLECTION AND EVALUATION

Data questionnaires (see Appendix) will be used both at the initial patient registration and at the follow-up evaluations. Data are transmitted to the study subcenters/local coordinators by completing and delivering the questionnaires. The subcenter/local coordinator will be available for clinical questions of the treating physicians, for reviewing the data sheets for completeness and correctness, and for transferring the revised data sheets to the study reference center in Stockholm.

The final evaluation will be performed in the study reference center, in collaboration with the study subcenters. The study reference center in Stockholm will survey the data collection, perform the overall data input and prepare the final data evaluation. Access to common data from the study data base will be given only with the approval of the Scientific Committee and permission of the Board of the Histiocyte Society.

Study Reference Center
(Stockholm/Henter)

Study Subcenters

Germany/ Centr Europe/ Janka	Italy/ S Europe/ Aricò	Japan/ Asia/ Imashuku	Netherlands/ Benelux/ Egeler	Sweden/ Scandinavia/ Henter	UK/ Gt Britain/	USA/ Canada / HAA-New Jersey

In addition, there are local coordinators in Austria (Dr Minkov, Vienna), South-America (Dr Braier, Buenos Aires), and Spain (Dr Astigarraga, Bilbao). For addresses, see page 3.

The review of statistical analyses will be made by Dr Scott M Montgomery, Clinical Epidemiology Unit, Karolinska Hospital L1:00, SE – 171 76 Stockholm, Sweden; telephone: +46 - 8 5177 9325, facsimile: +46 - 8 5177 9304.

An independent Data Safety Monitoring Board (DSMB) composed of three international experts will monitor the progress of the study on ethical and scientific grounds, Drs Finn Wesenberg, Åke Jakobson and Jim Whitlock, for the duration of the study.

Follow-up reports

For evaluation of the initial therapy response, the Follow-up form 1 (see Appendix) is sent to the local subcenter soon after completion of the initial therapy. The next Follow-up reports are scheduled at 2, 6 and 12 months after onset of therapy and later once yearly, unless a SCT has been performed at that time. The Documentation Sheets for the Initial therapy, Continuation therapy week 9-24 and 25-40, respecively, are sent once completed.

If SCT has been performed, the SCT follow-up +100-days form is used, and thereafter the SCT+1-year form is delivered once yearly after SCT.

Endpoint

The endpoint in the study is survival. The initial intention is to have a minimum of a 4-yr recruitment. There will be yearly evaluations regarding the need of modifications.

BIOLOGICAL STUDIES

The HLH-94 had a number of associated biological studies, including analyses of NK cell and T cell cytotoxicity, preparation of DNA for genetic analyses, as well as EBV-associated studies. These studies have been successful and they have improved diagnostics and therapy, and increased the biological understanding of the disease as well as of normal human immune modulation. In order to improve the diagnoses, therapy and biological understanding further, participation in the biological studies associated with HLH-2004 is encouraged.

Recent studies have shown that the disease is associated with decreased apoptosis triggering (8, 67, 94). This causes the defect in the NK and T cell cytotoxicity that has been known for long (12, 16-22). Two underlying gene defects have been revealed, mutations in the perforin gene (9-15), which account for 20-40% of all affected FHL cases (10), and in the hMunc 13-4 gene (23). It is possible to identify individuals with perforin mutations by the use of flow cytometry for the perforin protein (12). Moreover, it has also recently been shown that the cytotoxicity defect can be grouped in four subtypes (21), and that group 3 patients will most likely need a SCT in order to survive (22).

The biological studies in HLH-2004 address these recent novel findings. The goals are to:

1. Study the correlation of genetic mutations and associated flow cytometry results.
2. Gather biological material in order to identify additional genetic defects.
3. Study genotype-phenotype associations.
4. Study the biological and clinical significance of cytotoxic subgroups.
5. Document the infectious triggering agent in genetic as well as secondary HLH.

It is therefore suggested to sample for genetic analysis including flow cytometry from the study patients. It is also suggested to sample for analysis of NK- and T cell cytotoxicity. The HLH-2004 Study Group does not provide any financial support for the above studies.

GENETIC AND EXPRESSION STUDIES

Analysis of perforin is part of routine diagnostics in HLH, using sequencing and/or flow cytometry. This can be performed at certain specified laboratories for patients in the study. Laboratories with this service (Dec 2003) are listed in the Appendix (page 19). Similary, analysis of hMunc 13-4 may also be part of routine diagnostics, but this is not yet (Dec 2003) the case.

Gathering of material in order to identify additional genetic defects is important with the aim to identify other genetic defects than perforin and hMunc 13-4 mutations that are responsible for the development of HLH. These studies can be performed at each respective center, or as a collaborate effort upon the decision of each participating center.

Having genotype data and phenotype data together will also make it possible to perform important studies on genotype-phenotype correlations. This may hopefully provide information of value for the clinical care in the future.

NK CELL AND CYTOTOXIC T CELL ACTIVITY STUDIES

Analysis of NK and cytotoxic T cell activity is part of routine diagnostics in HLH. This is not only of diagnostic value but it also appears that NK-cell activity may have a prognostic value (21, 22). NK cell activity can be analyzed at certain specified laboratories for patients in the study. Laboratories with this service (Dec 2003) are listed in the Appendix (page 19).

PUBLICATION

Publication of overall study data or projects arisen from the overall study population may be undertaken only with the agreement of the Study Committee.

Every subcenter or participating clinic may present and publish their own data and observations, except treatment results, related to HLH patients that this particular center or clinic has reported to the Study, at any time.

REFERENCES

1. Favara BE, Feller AC, Pauli M, Jaffe ES, Wiess LM, Arico M, Bucsky P, Egeler M, Elinder G, Gadner H, Gresik M, Henter J-I, Imashuku S, Janka-Schaub G, Jaffe R, Ladisch S, Nezelof C, Pritchard J. Contemporary classification of histiocytic disorders. Med Pediatr Oncol 1997; 29: 157-166.

2. Janka GE: Familial hemophagocytic lymphohistiocytosis. Eur J Pediatr 1983; 140: 221-230.

3. Arico M, Janka G, Fischer A, Henter J-I, Blanche S, Elinder G, Martinetti M, Rusca MP; for the FHL Study Group of the Histiocyte Society. Hemophagocytic lymphohistiocytosis: Diagnosis, treatment and prognostic factors. Report of 122 children from the international registry. Leukemia 1996 Feb;10(2):197-203.

4. Henter J-I, Arico M, Elinder G, Imashuku S, Janka G. Familial hemophagocytic lymphohistiocytosis (primary HLH). Hematol Oncol Clin North Am 1998 Apr;12(2):417-33.

5. Janka G, Elinder G, Imashuku S, Schneider M, Henter J-I. Infection- and malignancy-associated hemophagocytic syndromes: Secondary hemophagocytic lymphohistiocytosis. Hematol Oncol Clin North Am 1998 Apr;12(2):435-44.

6. Henter J-I, Elinder G, Öst Å, and the FHL Study Group of the Histiocyte Society. Diagnostic guidelines for hemophagocytic lymphohistiocytosis. Semin Oncol 1991; 18: 29-33

7. Arico M, Danesino C, Pende D, Moretta L. Pathogenesis of haemophagocytic lymphohistiocytosis. Br J Haematol. 2001 Sep;114(4):761-9.

8. Fadeel B, Orrenius S, Henter J-I. Induction of apoptosis and caspase activation in cells obtained from familial haemophagocytic lymphohistiocytosis patients. Br J Haematol 1999; 106 (2): 406-15.

9. Stepp SE, Dufourcq-Lagelouse R, Le Deist F, Bhawan S, Certain S, Mathew PA, Henter J-I, Bennett M, Fischer A, de Saint Basile G, Kumar V. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. Science 1999; 286: 1957-59.

10. Ericson KG, Fadeel B, Nilsson-Ardnor S, Söderhäll C, Samuelsson AC, Janka G, Schneider M, Gürgey A, Yalman N, Révész T, Egeler RM, Jahnukainen K, Storm-Mathiesen I, Haraldsson A, Poole J, de Saint Basile G, Nordenskjöld M, Henter J-I. Spectrum of perforin gene mutations in familial hemophagocytic lymphohistiocytosis. Am J Hum Genet 2001 Mar; 68(3): 590-597.

11. Clementi R, zur Stadt U, Savoldi G, et al. Six novel mutations in the PRF1 gene in children with haemophagocytic lymphohistiocytosis. J Med Genet 2001; 38: 643-6.

12. Kogawa K, Lee SM, Villanueva J, Marmer D, Sumegi J, Filipovich AH. Perforin expression in cytotoxic lymphocytes from patients with hemophagocytic lymphohistiocytosis and their family members. Blood 2002 Jan 1;99(1):61-6

13. Suga N, Takada H, Nomura A, Ohga S, Ishii E, Ihara K, Ohshima K, Hara T. Perforin defects of primary haemophagocytic lymphohistiocytosis in Japan. Br J Haematol 2002 Feb;116(2):346-9

14. Ueda I, Morimoto A, Inaba T, Yagi T, Hibi S, Sugimoto T, Sako M, Yanai F, Fukushima T, Nakayama M, Ishii E, Imashuku S. Characteristic perforin gene mutations of hemophagocytic lymphohistiocytosis patients in Japan. Br J Haematol 2003; 121: 503-510

15. Feldmann J, Le Deist F, Ouachee-Chardin M, Certain S, Alexander S, Quartier P, Haddad E, Wulffraat N, Casanova JL, Blanche S, Fischer A, de Saint Basile G. Functional consequences of perforin gene mutations in 22 patients with familial haemophagocytic lymphohistiocytosis. Br J Haematol 2002 Jun;117(4):965-72

16. Perez N, Virelizier J, Arenzana-Seisdedos F, Fischer A, Criscelli C. Impaired natural killer cell activity in lymphohistiocytosis syndrome. J Pediatr 1984; 104: 569-73

17. Aricò M, Nespoli L, Maccario R, Montagna D, Bonetti F, Caselli D, Burgio GR. Natural cytotoxicity impairment in familial haemophagocytic lymphohistiocytosis. Arch Dis Child 1988; 63: 292-96

18. Eife R, Janka GE, Belohradsky BH, Holtmann H. Natural killer cell function and interferon production in familial hemophagocytic lymphohistiocytosis. Pediatr Hematol Oncol 1989;6(3):265-72.

19. Kataoka Y, Todo S, Morioka Y, Sugie K, Nakamura Y, Yidoi J, Imashuku S. Impaired natural killer activity and expression of interleukin-2 receptor antigen in familial erythrophagocytic lymphohistiocytosis. Cancer 1990; 65: 1937-41

20. Sullivan KE, Delaat CA, Douglas SD, Filipovich AH. Defective natural killer cell function in patients with hemophagocytic lymphohistiocytosis and in first degree relatives. Pediatr Res 1998 Oct; 44(4): 465-8.

21. Schneider EM, Lorenz I, Müller-Rosenberger M, Gerald Steinbach, Kron M, Janka G. Hemophagocytic lymphohistiocytosis (HLH) is associated with deficiencies of cellular cytolysis but normal expression of transcripts relevant to killer cell induced apoptosis. Blood 2002; 100: 2891-8.

22. Zheng C, Schneider EM, Samuelsson-Horne AC, Löfstedt M, Lorenz I, Janka G, Henter J-I. Natural killer cell activity subtypes provide therapeutic guidance in hemophagocytic lymphohistiocytosis. Abstr: 19:th Annual Meeting of the Histiocyte Society, Philadelphia, U S A, Sept 2003.

23. Feldmann J, Callebaut I, Raposo G, Certain S, Bacq D, Dumont C, Lambert N, Ouachee-Chardin M, Chedeville G, Tamary H, Minard-Colin V, Vilmer E, Blanche S, Le Deist F, Fischer A, de Saint Basile G. Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). Cell. 2003 Nov 14;115(4):461-73.

24. Henter J-I, Söder O, Öst Å, Elinder G. Incidence and clinical features of familial hemophagocytic lymphohistiocytosis in Sweden. Acta Paediatr Scand 1991;80:428-35

25. Risdall RJ, McKenna RW, Nesbit ME, Krivit W, Balfour HH Jr, Simmons RL, Brunning RD. Virus-associated hemophagocytic syndrome. A benign histiocytic proliferation distinct from malignant histiocytosis. Cancer 1979; 44: 993-1002

26. Arico M, Allen M, Brusa S, Clementi R, Pende D, Maccario R, Moretta L, Danesino C. Haemophagocytic lymphohistiocytosis: proposal of a diagnostic algorithm based on perforin expression. Br J Haematol. 2002 Oct;119(1):180-8.

27. Henter J-I, Ehrnst A, Andersson J, Elinder G. Familial hemophagocytic lymphohistiocytosis and viral infections. Acta Paediatr 1993; 82: 369-372.

28. Henter J-I, Arico M, Egeler M, Elinder G, Filipovich A, Favara B, Gadner H, Imashuku S, Janka G, Komp D, Ladisch S, Webb D. HLH-94: A treatment protocol for hemophagocytic lymphohistiocytosis. Med Pediatr Oncol 1997; 28: 342-47.

29. Henter J-I, Samuelsson-Horne AC, Arico M, Egeler RM, Elinder G, Filipovich AH, Gadner H, Imashuku S, Komp D, Ladisch S, Webb D, Janka G. Treatment of hemophagocytic lymphohistiocytosis with HLH-94 immuno-chemotherapy and bone marrow transplantation. Blood 2002, 100: 2367-2373.

30. Henter J-I, Horne AC, Aricò M, Egeler RM, Gadner H, Imashuku S, Janka G, Ladisch S, Montgomery SM, Webb D, Winiarski J, Filipovich AH. Hematopoietic stem cell transplantation in 90 children with hemophagocytic lymphohistiocytosis (HLH): Results of the HLH-94 Study. Blood 2002; 100 (11); abstract 144.

31. Esumi N, Ikushima S, Todo S, Imashuku S. Hyperferritinemia in malignant histiocytosis, virus-associated hemophagocytic syndrome and familial hemophagocytic lymphohistiocytosis. Acta Paediatr Scand 1989; 78: 268-270

32. Henter J-I, Elinder G. Familial hemophagocytic lymphohistiocytosis. Clinical review based on the findings in seven children. Acta Paediatr Scand 1991 Mar;80(3):269-77.

33. Favara B. Hemophagocytic lymphohistiocytosis: A hemophagocytic syndrome. Semin Diagn Pathol 1992; 9: 63-74

34. Henter J-I, Carlson LA, Nilsson-Ehle P, Söder O, Elinder G. Lipoprotein alterations and plasma lipoprotein lipase reduction in familial hemophagocytic lymphohistiocytosis. Acta Paediatr Scand 1991; 80: 675-81

35. Komp DM, McNamara J, Buckley P. Elevated soluble interleukin-2 receptor in childhood hemophagocytic histiocytic syndromes. Blood 1989; 73: 2128-32

36. Henter J-I, Elinder G, Söder O, Hansson M, Andersson B, Andersson U. Hypercytokinemia in familial hemophagocytic lymphohistiocytosis. Blood 1991; 78: 2918-2922

37. Howells DW, Strobel S, Smith I, Levinsky RJ, Hyland K. Central nervous system involvement in the erythrophagocytic disorders of infancy: The role of cerebrospinal fluid neopterins in their differential diagnosis and clinical management. Pediatr Res 1990; 28: 116-19

38. Imashuku S, Ikushima S, Esumi N, Todo S, Saito M. Serum levels of interferon-gamma, cytotoxic factor and soluble interleukin-2 receptor in childhood hemophagocytic syndrome. Leuk Lymphoma 1991; 3: 287-92.

39. Fujiwara F, Hibi S, Imashuku S. Hypercytokinemia in hemophagocytic syndrome. Amer J Pediatr Hematol Oncol 1993; 15: 92-98

40. Imashuku S, Hibi S, Fujiwara F, Ikushima S, Todo S. Hemophagocytic lymphohistiocytosis, interferon-gamma-nemia and Epstein-Barr virus involvement. Br J Haematol 1994; 88: 656-658.

41. Imashuku S, Hibi S, Sako M, Ishida Y, Mugishima H, Chen J, Tsunematsu Y. Soluble interleukin-2 receptor: a useful prognostic factor for patients with hemophagocytic lymphohistiocytosis. Blood 1995 Dec 15;86(12):4706-7.

42. Seemayer TA, Grierson H, Pirruccello SJ, Gross TG, Weisenburger DD, Davis J, Spiegel K, Brichacek B, Sumegi J. X-linked lymphoproliferative disease. Am J Dis Child 1993; 147: 1242-45.

43. Nichols KE. X-linked lymphoproliferative disease: genetics and biochemistry. Rev Immunogenet. 2000;2(2):256-66.

44. Grunebaum E, Zhang J, Dadi H, Roifman CM. Haemophagocytic lymphohistiocytosis in X-linked severe combined immunodeficiency. Br J Haematol 2000 Mar;108(4):834-7

45. Arico M, Imashuku S, Clementi R, et al. Hemophagocytic lymphohistiocytosis due to germline mutations in SH2D1A, the X-linked lymphoproliferative disease gene. Blood 2001; 97: 1131-1133

46. Sumegi J, Seemayer TA, Huang D, Davis JR, Morra M, Gross TG, Yin L, Romco G, Klein E, Terhorst C, Lanyi A. A spectrum of mutations in SH2D1A that causes X-linked lymphoproliferative disease and other Epstein-Barr virus-associated illnesses. Leuk Lymphoma 2002 Jun;43(6):1189-201.

47. Ward DM, Shiflett SL, Kaplan J. Chediak-Higashi syndrome: a clinical and molecular view of a rare lysosomal storage disorder. Curr Mol Med 2002 Aug;2(5):469-77.

48. Sanal O, Ersoy F, Tezcan I, Metin A, Yel L, Menasche G, Gurgey A, Berkel I, de Saint Basile G. Griscelli disease: genotype-phenotype correlation in an array of clinical heterogeneity. J Clin Immunol 2002 Jul;22(4):237-43

49. Duval M, Fenneteau O, Doireau V, Faye A, Emilie D, Yotnda P, Drapier JC, Schlegel N, Sterkers G, de Baulny HO, Vilmer E. Intermittent hemophagocytic lymphohistiocytosis is a regular feature of lysinuric protein intolerance. J Pediatr 1999 Feb;134(2):236-9.

50. Schmid I, Reiter K, Schuster F, Wintergerst U, Meilbeck R, Nicolai T, Behloradsky BH, Stachel DK. Allogeneic bone marrow transplantation for active Epstein-Barr virus-related lymphoproliferative disease and hemophagocytic lymphohistiocytosis in an infant with severe combined immunodeficiency syndrome. Bone Marrow Transplant 2002 Mar;29(6):519-21

51. Aleman K, Noordzij JG, de Groot R, van Dongen JJ, Hartwig NG. Reviewing Omenn syndrome. Eur J Pediatr 2001 Dec;160(12):718-25.

52. Imashuku S, Hibi S, Ohara T, Iwai A, Sako M, Kato M, Arakawa H, Sotomatsu M, Kataoka S, Asami K, Hasegawa D, Kosaka Y, Sano K, Igarashi N, Maruhashi K, Ichimi R, Kawasaki H, Maeda N, Tanizawa A, Arai K, Abe T, Hisakawa H, Miyashita H, Henter J-I. Effective control of Epstein-Barr virus-related hemophagocytic lymphohistiocytosis with immunochemotherapy. Blood 1999; 93: 1869-74.

53. Lichtenheld MG, Podack ER. Structure of the human perforin gene. A simple gene organization with interesting potential regulatory sequences. J Immunol 1989; 143:4267-4274

54. Tschopp J, Nabholz M. Perforin-mediated target cell lysis by cytolytic T lymphocytes. Annu Rev Immunol 1990; 8:279-302

55. Lowin B, Peitsch MC, Tschopp J. Perforin and granzymes: crucial effector molecules in cytolytic T lymphocyte and natural killer cell-mediated cytotoxicity. Curr Top Microbiol Immunol 1995; 198:1-24

56. Darmon AJ, Nicholson DW, Bleackley RC. Activation of the apoptotic protease CPP32 by cytotoxic T-cell-derived granzyme B. Nature 1995; 377:446-44

57. Darmon AJ, Bleackley RC. Proteases and cell-mediated cytotoxicity. Crit Rev Immunol 1998; 18:255-273

58. Pinkoski MJ, Green DR. Lymphocyte apoptosis: refining the paths to perdition. Curr Opin Hematol 2002; 9: 43-49.

59. Henter J-I, Elinder G. Cerebromeningeal hemophagocytic lymphohistiocytosis. Lancet 1992; i: 104-07

60. Schneider EM, Janka G. Hemophagocytic lymphohistiocytosis (HLH)-state of the art. http://www.histio.org/society/HLH/janka1.shtml

61. Allen M, De Fusco C, Legrand F, Clementi R, Conter V, Danesino C, Janka G, Aricó M. Familial hemophagocytic lymphohistiocytosis: how late can the onset be? Haematologica 2001 May; 86 (5): 499-503

62. Clementi R, Emmi L, Maccario R, Liotta F, Moretta L, Danesino C, Arico M. Adult onset and atypical presentation of hemophagocytic lymphohistiocytosis in siblings carrying PRF1 mutations. Blood. 2002 Sep 15;100(6):2266-7.

63. Ladisch S, Ho W, Matheson D, Pilkington R, Hartman G. Immunologic and clinical effects of repeated blood exchange in familial erytrophagocytic lymphohistiocytosis. Blood 1982; 60: 814-21

64. Ambruso DR, Hays T, Zwartjes WJ, Tubergen DG, Favara BE: Successful treatment of lymphohistiocytic reticulosis with phagocytosis with epipodophyllotoxin VP 16-213. Cancer 1980; 45: 2516-2520

65. Henter J-I, Elinder G, Finkel Y, Söder O. Successful induction with chemotherapy including teniposide in familial erythrophagocytic lymphohistiocytosis. Lancet 1986; ii: 1402

66. Fischer A, Virelizier JL, Arenzana-Seisdedos F, Perez A, Nezelof C, Griscelli C. Treatment of four patients with erythrophagocytic lymphohistiocytosis by a combination of epipodophyllotoxin, steroids, intrathecal methotrexate and cranial irradiation. Pediatrics 1985; 76: 263-268

67. Fadeel B, Orrenius S, Henter J-I. Familial hemophagocytic lymphohistiocytosis: too little cell death may seriously damage your health. Leuk Lymphoma 2001; 42: 13-20.

68. Fischer A, Cerf-Bensussan N, Blanche S, LeDeist F, Bremard-Oury C, Leverger G, Schaison G, Durandy A, Griscelli C. Allogeneic bone marrow transplantation for erythrophagocytic lymphohistiocytosis. J Pediatr 1986; 108: 267-70

69. Todo S, Fujiwara F, Ikushima S, Yoshihara T, Kataoka Y, Sakurai-Shimada Y, Sakata K, Kasubuchi Y, Morioka Y, Imashuku S. Allogeneic bone marrow transplantation for familial erythrophagocytic lymphohistiocytosis with high dose VP16-containing conditioning regimen. Leuk Lymphoma 1990; 1: 361-64

70. Blanche S, Caniglia M, Girault D, Landman J, Griscelli C, Fischer A. Treatment of hemophagocytic lymphohistiocytosis with chemotherapy and bone marrow transplantation: a single center study of 22 cases. Blood 1991; 78: 51-54

71. Nespoli L, Locatelli F, Bonetti F, Porta F, Zecca M, Gibardi A, Gambarana D, Vitale V, Burgio GR, Severi F. Familial hemophagocytic lymphohistiocytosis treated with allogeneic bone marrow transplantation. Bone Marrow Transplant 1991; 7 Suppl 3: 139-42

72. Bolme P, Henter J-I, Winiarski J, Elinder G, Ljungman P, Lönnerholm G, Ringdén O. Allogeneic bone marrow transplantation in hemophagocytic lymphohistiocytosis in Sweden. Bone Marrow Transplant 1995; 15: 331-35

73. Baker KS, DeLaat CA, Steinbuch M, et al. Successful correction of hemophagocytic lymphohistiocytosis with related or unrelated bone marrow transplantation. Blood 1997; 89: 3857-3863.

74. Jabado N, de Graeff-Meeder ER, Cavazzana-Calvo M, et al. Treatment of familial hemophagocytic lymphohistiocytosis with bone marrow transplantation from HLA genetically nonidentical donors. Blood 1997; 90: 4743-4748.

75. Imashuku S, Hibi S, Todo S, Sako M, Inoue M, Kawa K, Koike K, Iwai A, Tsuchiya S, Akiyama Y, Kotani T, Kawamura Y, Hirosawa M, Hasegawa D, Kosaka Y, Yamaguchi H, Ishii E, Kato K, Ishii M, Kigasawa H. Allogeneic hematopoietic stem cell transplantation for patients with hemophagocytic syndrome (HPS) in Japan. Bone Marrow Transplant 1999 Mar;23(6):569-72

76. Dürken M, Horstmann M, Bieling P, et al. Improved outcome in haemophagocytic lymphohistiocytosis after bone marrow transplantation from related and unrelated donors: a single-centre experience of 12 patients. Br J Haematol 1999; 106:1052-1058

77. Akima M, Sumi SM. Neuropathology of familial erythrophagocytic lymphohistiocytosis. Six cases and review of the literature. Hum Pathol 1984;15:161-68

78. Haddad E, Sulis ML, Jabado N, Blanche S, Fischer A, Tardieu M. Frequency and severity of central nervous system lesions in hemophagocytic lymphohistiocytosis. Blood 1997; 89: 794-800

79. Henter J-I, Nennesmo I. Neuropathological findings and neurological symptoms in 23 children with hemophagocytic lymphohistiocytosis. J Pediatr 1997; 130: 358-365.

80. Imashuku S, Hyakuna N, Funabiki T, Ikuta K, Sako M, Iwai A, Fukushima T, Kataoka, S, Yabe M, Muramatsu K, Kohdera U, Nakadate H, Kitazawa K, Toyoda Y, Ishii E. Low natural killer activity and central nervous system disease as a high-risk prognostic indicator in young patients with hemophagocytic lymphohistiocytosis. Cancer 94; 3023-3031: 2002

81. Loechelt BJ, Egeler RM, Filipovich AH, Jyonouchi H, Shapiro RS. Immunosuppression: an alternative maintenance therapy for familial hemophagocytic lymphohistiocytosis (FHL): Preliminary results. Med Pediatr Oncol 22: 325-28, 1994

82. Stephan JL, Donadieu J, Ledeist F, Blanche S, Griscelli and Fischer A. Treatment of familial hemophagocytic lymphohistiocytosis with antithymocyte globulins, steroids and cyclosporin A. Blood 1993; 82: 2319-2323

83. Imashuku S, Kuriyama K, Teramura T, Ishii E, Kinugawa N, Kato M, Sako M, Hibi S. Requirement for etoposide in the treatment of Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis. J Clin Oncol 2001 May 15;19(10):2665-73.

84. Imashuku S, Ikushima S, Hibi S, Todo S. Langerhans cell histiocytosis and hemophagocytic syndrome in Japan; epidemiological studies. Int J Pediatr Hematol Oncol 1994; 1: 241-46

85. Athreya BH. Is macrophage activation syndrome a new entity? Clin Exp Rheumatol. 2002 Mar-Apr;20(2):121-3.

86. Ramanan AV, Baildam EM. Macrophage activation syndrome is hemophagocytic lymphohistiocytosis--need for the right terminology. J Rheumatol. 2002 May;29(5):1105.

87. Stephan JL, Kone-Paut I, Galambrun C, Mouy R, Bader-Meunier B, Prieur AM. Reactive haemophagocytic syndrome in children with inflammatory disorders. A retrospective study of 24 patients. Rheumatology (Oxford) 2001;40:1285-92.

88. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, LernerKG, Thomas ED. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation 1974;18(4):295-304.

89. Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE, Hackman R, Tsoi MS, Storb R, Thomas ED. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med 1980 Aug;69(2):204-17

90. McDonald GB, Hinds MS, Fisher LD, Schoch HG, Wolford JL, Banaji M, Hardin BJ, Shulman HM, Clift RA. Veno-occlusive disease of the liver and multiorgan failure after bone marrow transplantation: a cohort study of 355 patients. Ann Intern Med 1993 Feb 15;118(4):255-67

91. Bearman SI, Appelbaum FR, Buckner CD, Petersen FB, Fisher LD, Clift RA, Thomas ED. Regimen-related toxicity in patients undergoing bone marrow transplantation. J Clin Oncol 1988 Oct;6(10):1562-8

92. Henter J-I, Elinder G, Lübeck P-O, Öst Å. Myelodysplastic syndrome following epipodophyllotoxin therapy in familial hemophagocytic lymphohistiocytosis. Pediatr Hematol Oncol 1993; 10: 163-68.

93. Imashuku S, Teramura T, Kuriyama K, Kitazawa J, Ito E, Morimoto A, Hibi S. Risk of etoposide-related acute myeloid leukemia in the treatment of Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis. Int J Hematol 2002 Feb;75(2):174-7

94. Henter J-I. Biology and treatment of familial hemophagocytic lymphohistiocytosis: importance of perforin in lymphocyte-mediated cytotoxicity and triggering of apoptosis. Med Pediatr Oncol 2002; 38(5): 305-309.