EUROPEAN HEMATOLOGY ASSOCIATION
haematologica
Journal of the European Hematology Association

The syndrome of hemophagocytic lymphohistiocytosis in primary immunodeficiencies: implications for differential diagnosis and pathogenesis

by Sebastian F.N. Bode, Sandra Ammann, Waleed Al-Herz, Mihaela Bataneant, Christopher C. Dvorak, Stephan Gehring, Andrew Gennery, Kimberley C. Gilmour, Luis I. Gonzalez-Granado, Ute Groß-Wieltsch, Marianne Ifversen, Jenny Lingman-Framme, Susanne Matthes-Martin, Rolf Mesters, Isabelle Meyts, Joris M. van Montfrans, Jana Pachlopnik Schmid, Sung-Yun Pai, Pere Soler-Palacin, Uta Schuermann, Volker Schuster, Markus G. Seidel, Carsten Speckmann, Polina Stepensky, Karl-Walter Sykora, Bianca Tesi, Thomas Vraetz, Catherine Waruiru, Yenan T. Bryceson, Despina Moshous, Kai Lehmberg, Michael B. Jordan, and Stephan Ehl

Haematologica 2015 [Epub ahead of print]

Citation: Bode SF, Ammann S, Al-Herz W, Bataneant M, Dvorak CC, Gehring S, Gennery A, Gilmour KC, Gonzalez-Granado LI, Groß-Wieltsch U, Ifversen M, Lingman-Framme J, Matthes-Martin S, Mesters R, Meyts I, van Montfrans JM, Schmid J, Pai SY, Soler-Palacin P, Schuermann U, Schuster V, Seidel MG, Speckmann C, Stepensky P, Sykora KW, Tesi B, Vraetz T, Waruiru C, Bryceson YT, Moshous D, Lehmberg K, Jordan MB, and Ehl S. The syndrome of hemophagocytic lymphohistiocytosis in primary immunodeficiencies: implications for differential diagnosis and pathogenesis. Haematologica. 2015; 100:xxx
doi:10.3324/haematol.2014.121608

The syndrome of hemophagocytic lymphohistiocytosis in primary immunodeficiencies: implications for differential diagnosis and pathogenesis

Sebastian F.N. Bode[1,2], Sandra Ammann[1,3], Waleed Al-Herz[4], Mihaela Bataneant[5], Christopher C. Dvorak[6], Stephan Gehring[7], Andrew Gennery[8], Kimberly C. Gilmour[9], Luis I. Gonzalez-Granado[10], Ute Groß-Wieltsch[11], Marianne Ifversen[12], Jenny Lingman-Framme[13], Susanne Matthes-Martin[14], Rolf Mesters[15], Isabelle Meyts[16], Joris M. van Montfrans[17], Jana Pachlopnik Schmid[18], Sung-Yun Pai[19], Pere Soler-Palacin[20], Uta Schuermann[21], Volker Schuster[22], Markus G. Seidel[23], Carsten Speckmann[1,2], Polina Stepensky[24], Karl-Walter Sykora[25], Bianca Tesi[26], Thomas Vraetz[2], Catherine Waruiru[27], Yenan T. Bryceson[28], Despina Moshous[29], Kai Lehmberg[30], Michael B. Jordan[31*] and Stephan Ehl[1,2*] for the Inborn Errors Working Party of the EBMT.

1Centre of Chronic Immunodeficiency, University Medical Centre Freiburg, Freiburg, Germany;

2Centre for Pediatrics and Adolescent Medicine, University Medical Centre Freiburg, Germany;

3Faculty of Biology, University of Freiburg, Germany

4Department of Pediatrics, Faculty of Medicine, Kuwait University, Safat, Kuwait;

5Discipline of Pediatrics III, Victor Babes University of Medicine and Pharmacy Timisoara, Timisoara, Romania;

6Pediatric Allergy, Immunology and Blood and Marrow Transplant Division, UCSF, Benioff Children's Hospital, San Francisco, California, USA;

7Centre for Pediatrics and Adolescent Medicine, Mainz, Germany;

8Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom;

9Camelia Botnar Laboratories, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom;

10Immunodeficiencies Unit, Hematology & Oncology Unit, Pediatrics, Hospital 12 Octubre, Madrid, Spain;

11Pediatric Hematology, Oncology and Immunology, Olga Hospital, Stuttgart, Germany;

12Department of Pediatrics, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark

13Department of Pediatrics, Halland Hospital, Halmstad, Sweden;

14St Anna Children's Hospital, Vienna, Austria;

15Department of Medicine/Hematology and Oncology, University Hospital Muenster, Muenster, Germany;

16Department of Pediatrics, Department of Micriobiology and Immunology, University Hospitals Leuven, Katholieke Universiteit Leuven, Leuven, Belgium;

17Department of Pediatric Immunology, Wilhelmina Children's, Hospital/University Medical Centre Utrecht, Utrecht, The Netherlands;

18Jeffrey Modell Diagnostic Center for Primary Immunodeficiencies, University Children's Hospital Zuerich, Zuerich, Switzerland;

19Division of Hematology-Oncology, Boston Children's Hospital and Department of Pediatric Oncology, Dana-Farber Children's Hospital; Boston, Massachusetts, USA;

20Pediatric Infectious Diseases and Immunodeficiencies Unit. Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain;

21Children's Hospital of Datteln, University of Witten-Herdecke, Datteln, Germany;

22Hospital for Children and Adolescents, University of Leipzig, Germany;

23Pediatric Hematology-Oncology, Medical University of Graz, Austria;

24Pediatric Hematology-Oncology and Bone Marrow Transplantation, Hadassah Hebrew University Hospital, Jerusalem, Israel;

25Pediatric Hematology-Oncology, Medical School Hannover, Hanover, Germany;

26Childhood Cancer Research Unit, Department of Women's and Children's Health, Karolinska Institutet, Karolinska University Hospital Solna, Stockholm, Sweden;

27Sheffield Children's Hospital, NHS Foundation Trust, Sheffield, United Kingdom;

28Centre for Infectious Medicine, Department of Medicine, Karolinska Institutet, University Hospital Huddinge, Stockholm, Sweden

29Unit for Pediatric Immunology, Hematology and Rheumatology (UIHR), Hôpital Necker-Enfants Malades, Paris, France;

30Department of Haematology and Oncology, Children's Hospital, University of Hamburg, Hamburg, Germany.

31Division of Bone Marrow Transplantation and Immunodeficiency, Cincinnati Children's Hospital Medical Center, University of Cincinnati Medical School, Cincinnati, Ohio, USA;

*These two authors contributed equally to this work.

Corresponding author:

Prof. Dr. Stephan Ehl

Centre of Chronic Immunodeficiency

University Medical Centre Freiburg

Freiburg

Germany

Mail: stephan.ehl@uniklinik-freiburg.de

Phone: +49-761-270-77550

Abstract

Hemophagocytic lymphohistiocytosis is a hyperinflammatory syndrome defined by clinical and laboratory criteria. Current criteria were created to identify patients with familial hemophagocytic lmyphohistiocytosis in immediate need of immunosuppressive therapy. However, these criteria also identify patients with infection-associated hemophagocytic inflammatory states lacking genetic defects typically predisposing to hemophagocytic lymphohistiocytosis. This includes patients with primary immunodeficiencies, in whom pathogenesis of the inflammatory syndrome may be distinctive and aggressive immunosuppression contraindicated. To better characterize hemophagocytic inflammation associated with immunodeficiencies, we combined an international survey with a literature search and identified 63 patients with primary immunodeficiencies other than cytotoxicity defects or X-linked lymphoproliferative disorders, presenting with conditions fulfilling current criteria for hemophagocytic lymphohistiocytosis. Twelve patients had severe combined immunodeficiency with <100/µl T-cells, 18 had partial T-cell deficiencies; episodes of hemophagocytic lymphohistiocytosis were mostly associated with viral infections. Twenty-two patients had chronic granulomatous disease with hemophagocytic episodes mainly associated with bacterial infections. Compared to patients with cytotoxicity defects, patients with T-cell deficiencies had lower soluble CD25 and higher ferritin. Other criteria for hemophagocytoc lymphohistiocytosis were not discriminative. Thus, (i) a hemophagocytic inflammatory syndrome fulfilling criteria for hemophagocytic lymphohistiocytosis can be the initial manifestation of primary immunodeficiencies. (ii) this syndrome can develop despite severe deficiency of T- and NK-cells, implicating that pathophysiology is distinct and not appropriately described as “Lympho”-Histiocytosis in these patients (iii) current criteria for hemophagocytoc lymphohistiocytosis are insufficient to differentiate hemophagocytic inflammatory syndromes with different pathogenesis. This is important because of implications for therapy, in particular for protocols targeting T-cells.

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening hyperinflammatory syndrome. The term was initially coined based on histomorphological features.[1] The hereditary disorders, in which the HLH syndrome is the defining clinical manifestation, have been associated with autosomal recessive mutations in genes encoding perforin (familial hemophagocytic lymphohistiocytosis, FHL-2) and a group of proteins required for secretion of perforin-containing cytotoxic granules (FHL 3-5, Griscelli syndrome type 2 and Chediak-Higashi syndrome).[1] HLH is also a frequent manifestation of some defined genetic disorders of EBV susceptibility, *e.g.* X-linked lymphoproliferative syndromes (XLP1 and XLP2).[2] Impaired lymphocyte cytotoxicity with highly activated, but inefficient T cells are the main pathogenic factors in the former group of disorders,[3] while the pathophysiological basis of HLH in XLP and other syndromes of EBV susceptibility is less well understood.

As defined by the Histiocyte Society, the diagnosis of the HLH syndrome is based on fulfillment of five out of eight clinical and laboratory parameters or a molecular diagnosis of a disease conferring a high risk of developing HLH. These criteria have been useful for identification of patients with genetic defects in lymphocyte cytotoxicity. However, they are also fulfilled by a range of other patients presenting with hemophagocytic inflammatory disease, but normal cytotoxicity. Thus, the HLH syndrome can manifest in the context of severe infections including viral infections or sepsis/systemic inflammatory response syndrome, autoimmune and autoinflammatory diseases or malignancies such as lymphomas.[4-6] These disease states are frequently summarized as “secondary HLH”, “acquired HLH” or “macrophage activation syndrome”. Affected patients usually present with clinical and laboratory manifestations that cannot be readily distinguished from those observed in patients with defects in cytotoxicity.[4-6] However, the fact that defective cytotoxicity cannot be consistently found in such patients suggests that the pathophysiological pathways leading to the HLH syndrome may differ between different groups of patients.[5]

Current treatment guidelines based on the HLH-2004 study of the Histiocyte Society recommend that HLH-directed therapy should be strongly considered if five out of eight diagnostic criteria are fulfilled, irrespective of whether they occur in the presence of defects in lymphocyte cytotoxicity or in other forms of the disease.[7] While there is no doubt that this therapy can be life-saving in patients with FHL and many instances of infection-associated HLH,[8] less intensive anti-inflammatory treatment is frequently sufficient for patients with other forms of hemophagocytic inflammatory disease and aggressive immunosuppression may even be contraindicated.[1, 9] Moreover, more specific therapies for HLH targeting T cells[9] or IFN-γ[3,10] are undergoing prospective evaluation. Potential differences in the pathogenetic events leading to the HLH syndrome therefore become increasingly relevant.

One well-defined group of patients in whom the HLH syndrome has been described, are patients with primary immunodeficiencies (PID) other than FHL or XLP. Single cases or small case series of the HLH syndrome have been reported in a variety of PIDs, and the clinical presentation of some of these cases has recently been summarized.[11] However, a multicenter systematic analysis of the clinical and laboratory features of the HLH syndrome in these patients in comparison to HLH associated with defects in lymphocyte cytotoxicity has not been performed. We reasoned that such an analysis might offer the opportunity to identify parameters for differential diagnosis, facilitating early identification of patients with hemophagocytic inflammatory disease in which aggressive immunosuppressive therapy may be contraindicated. Furthermore, since primary immunodeficiencies provide an excellent possibility to study the role of constitutional immunological abnormalities in immunopathological conditions, we expected to gain some insights relevant to the pathogenesis of the HLH syndrome.

Methods

Patient recruitment

To identify patients with PID other than disorders of cytotoxicity or XLP and a clinical presentation with a disease state fulfilling the HLH-2004 criteria for HLH, we performed a survey among centers involved in the diagnosis and treatment of PID and/or HLH through the Histiocyte Society, the European Society of Blood and Bone Marrow Transplantation's Inborn Errors Working Party and the German Society for Pediatric Oncology and Hematology. These data were supplemented by a literature review based on a PubMed search for "hemophagocytic lymphohistiocytosis", "macrophage activation syndrome", and "immunodeficiency" or individual immunodeficiencies such as "chronic granulomatous disease" (CGD) up to December 31st, 2014. All cases were reviewed by SFNB and SE. Patients were included if they fulfilled the following criteria: (i) genetic or clinical diagnosis of a defined PID as classified by the international union of immunological societies expert committee for primary immunodeficiency (IUIS) except for genetic defects in cytotoxicity, XLP1 or XLP2.[12] (ii) Fulfillment of at least five out of eight diagnostic criteria for HLH according to the HLH-2004 criteria.[7] These include: 1. fever, 2. splenomegaly, 3. cytopenia of ≥2 cell lines (hemoglobin ≤90 g/L, platelets ≤100 x10^9/L, neutrophils ≤1 x10^9/L), 4. hypofibrinogenemia (≤1.5 g/L) or hypertriglyceridemia (≥265 mg/dL), 5. hyperferritinemia (≥500 ng/mL), 6. increased level of soluble CD25 (sCD25, ≥2400 U/mL), 7. evidence of hemophagocytosis, and 8. decreased or absent NK-cell cytotoxicity. From all patients, we recorded the underlying immunodeficiency diagnosis, the individual diagnostic criteria for HLH, associated infections, treatment and outcome of the HLH episode and whether or not HLH developed at/before or after PID diagnosis.

Control groups

Laboratory values of these patients were compared to those observed in a cohort of patients with active "primary" HLH (FHL 2-5; n=90) and a cohort of patients with infection-associated "secondary" HLH (I-HLH; n=40) without another known underlying disease such as

malignancy, rheumatological or metabolic disease from the German HLH registry. This registry collects clinical, immunological and genetic information on patients referred for evaluation for HLH from German speaking countries. Around 100 patients are referred each year and around 60 of these fulfill the diagnostic criteria for HLH. The group of patients with I-HLH consisted of patients who (i) fulfilled at least 5/8 diagnostic criteria for HLH, (ii) had an infection proven by positive PCR or unequivocal serology at the time of HLH manifestation, (iii) had neither a mutation in HLH-related genes (if investigated) or another obvious underlying genetic disease nor a positive family history for HLH and (iv) did not have disease relapse within at least 12 months after HLH manifestation.[13] About 20% of the patients in this group were thoroughly evaluated for mutations in HLH-causing genes because of ambiguous or abnormal results in the functional analysis.

Statistical analysis

Statistical analysis was performed with the GraphPad InStat software version 6.05. First we calculated the natural logarithms of the mean of sCD25, ferritin, fibrinogen, and triglycerides. Differences between group means were then evaluated with a one-way analysis of variance with post-hoc Tukey test. Differences were considered significant at $p<0.05$.

Ethics

The study was approved by the ethics committee of the Albert-Ludwigs-Universität Freiburg (AK28/14) and conducted according to the Declaration of Helsinki.

Results

Patient cohort and sequence of diagnosis

We identified 28 patients with primary immunodeficiencies other than defects in cytotoxicity or XLP fulfilling the HLH-2004 diagnostic criteria through the survey and found 30 publications describing another 35 cases (Table 1).[14-43] Six of the 18 cases recently summarized by Faitelson[11] were not included in this analysis because of incomplete information on our inclusion criteria as were 10 cases identified by either the survey or the literature review. In total, we identified 63 patients with PID and an HLH syndrome. Median age at diagnosis of the HLH syndrome was 1.5 years (range 0.13-34 years). Notably, an HLH syndrome was the initial presentation leading to diagnosis of the underlying PID in 36 patients, whereas 25 patients developed HLH after the PID diagnosis had been established; for two patients this information was not available.

Spectrum of primary immunodeficiencies presenting with an HLH syndrome

Two main groups of PID represented more than 80% of patients. Thirty patients had combined immunodeficiencies (CID). More specifically, twelve patients had SCID with a variety of molecular causes (Table 1). Interestingly, in 9/12 of these patients, the HLH syndrome developed in the first months of life, at or before the diagnosis of the immunodeficiency. Eighteen patients had other well-characterized combined immunodeficiencies affecting T cell development or function, including “atypical” SCID[44] with significant numbers of autologous T cells (n=2), 22q11 deletion syndrome (n=4), Wiskott-Aldrich syndrome (n=4), ataxia telangiectasia (n=1) and dyskeratosis congenita (n=1). Two patients had CD27 deficiency and one had *ITK* deficiency, conditions that have previously been associated with poor control of EBV infection (Table 1). [45, 46] The second main group consisted of 22 patients with chronic granulomatous disease (CGD) (Table 1). Most of these patients presented with an HLH syndrome beyond the first year of life, some in adulthood. In nine of the younger patients (<4 years), the HLH syndrome developed at or before the diagnosis of CGD. Only eleven patients had other PID, including antibody deficiencies (n=2),

diseases of immune dysregulation (n=2), other congenital defects of phagocytes (n=1), defects in innate immunity (n=2), or autoinflammatory disorders (n=4) (Table 1).

Infections associated with the HLH syndrome in PID patients

In 50/63 patients (79%), the HLH syndrome was associated with an infection. In SCID and CID patients, it was mainly associated with viral infections. Ten of 30 (33%) patients had EBV infection, 7 had CMV infection, three had infections with adenovirus and two had infections with other gastrointestinal or respiratory viruses. In contrast, in only two of 22 (9%) of CGD patients, the HLH syndrome was associated with a viral infection and in both cases an additional bacterial infection was diagnosed. The main infectious agents in the group of CGD patients were *Burkholderia cepacia* (n=7), *Leishmania spp.* (n=6), and fungi (n=4). In the group of eleven patients with other PID, the HLH syndrome was associated with a viral infection in three and a bacterial infection in two patients (table 1).

Lymphocyte compartment in SCID patients

Since in familial HLH (FHL), activated T cells and possibly NK cells are regarded as major effectors in disease pathogenesis,[1, 3] we had a closer look at the lymphocyte compartment in the SCID patients. Interestingly, all SCID patients had fewer than 100 T cells/µl (including 9 patients with less than 20 T cells/µl) (Table 2). Four patients had been analyzed for maternal T cells, which were detected at low numbers in 2 of them. Three patients also had 10 or fewer NK cells/µl. The number of B cells was more variable.

Diagnostic HLH parameters in PID patients presenting with the HLH syndrome

Information on all eight HLH-2004 diagnostic criteria was only available for 7 patients, while 7 criteria were reported in additional 15 patients. These patients usually lacked information on sCD25 (reported in 23/63 patients, 36%) or NK cell cytotoxicity (reported in 19/63 patients, 30%). On the other hand, all patients fulfilled at least 5 diagnostic criteria. In 36 patients (57%), all of the 5-8 reported criteria were positive. When looking at individual criteria, no particular pattern emerged that would be characteristic for PID or PID subgroups. Of the

patients for which information on these criterion was available, ninety-eight percent had fever, 91% had splenomegaly, 91% had cytopenias, 80% had elevated triglycerides, 75% had low fibrinogen, 100% had elevated ferritin, 82% had elevated sCD25 and 63% had low or absent NK cell cytotoxicity.

We then quantitatively compared the laboratory values of patients with T cell deficiencies and CGD to those obtained in a cohort with FHL (n=90) and a cohort of patients with infection-associated HLH without known underlying hereditary disease (I-HLH, n=40). The most significant differences were observed in the serum concentrations of soluble interleukin-2 receptor (sCD25) (Figure 1A, Table 3). Patients with T cell deficiencies had significantly lower values than those observed in patients with FHL (mean: T cell deficiencies: 5290 U/ml, FHL: 23074 U/ml; $p<0.0001$). Notably, levels were also lower in patients with I-HLH than in patients with FHL. In contrast, the concentrations of ferritin were higher in the patients with T cell deficiencies than in the other three patient groups, but only significantly higher when compared to FHL patients ($p<0.05$; mean: T cell deficiencies: 64808 ng/ml, FHL: 10154 ng/ml, I-HLH: 8359 ng/ml, CGD 8819 ng/ml; Figure 1B). These differences were even more obvious, when the ratio of ferritin/sCD25 was determined, which was significantly higher in patients with T cell deficiency compared to FHL, I-HLH, and CGD patients (mean: T cell deficiencies: 14.18, FHL: 0.37, I-HLH: 0.48, CGD: 0.90; Figure 1C; see figure for p-values). Apart from a significant difference in fibrinogen levels between FHL and I-HLH patients, no relevant differences were observed in the other HLH-defining parameters (mean FHL: 1.218 g/l, I-HLH: 1.869 g/l; $p<0.05$; Figure 2).

Treatment and outcome

The patients received a wide range of HLH-directed treatment regimens, including corticosteroids alone or in combination with IVIG, cyclosporine or rituximab (Table 1). Etoposide based therapy was given to 14 patients. This included 10 patients with T cell deficiencies, 6 of whom received their PID diagnosis during or after the episode of HLH syndrome. Only one patient with CGD received etoposide based therapy. Thirty-nine patients

(65%) of 60 patients with reported outcome survived the HLH syndrome. Of note, only 4/12 SCID patients and 9/18 patients with CID and reported outcome survived, which contrasted with 20/22 CGD patients.

Discussion

This study identified 63 patients with PID other than genetic disorders of cytotoxicity or XLP, who fulfilled the current clinical criteria for HLH. In 36 of them, this HLH syndrome was the initial manifestation before or at the diagnosis of PID. Primary immunodeficiencies other than disorders of cytotoxicity or XLP are therefore a relevant differential diagnosis in patients presenting with the clinical picture of the HLH syndrome.

While many PIDs may predispose to an infection-triggered HLH syndrome, this was largely restricted to two groups in this cohort: patients with T cell deficiencies, who are susceptible to a broad range of infections, virus infections in particular, and patients with chronic granulomatous disease, who are susceptible to a smaller spectrum of bacterial and opportunistic, but not to viral infections. Infections associated with the HLH syndrome in PID patients reflected this specific susceptibility, compatible with the notion that uncontrolled pathogen replication is a major risk factor for this hyperinflammatory syndrome. However, while the HLH syndrome was observed in a relevant number of CGD patients, it was not reported in other congenital defects in phagocytes and only rarely in other defects in innate immunity. It is therefore tempting to hypothesize that the HLH syndrome in CGD may not only reflect impaired infection control, but also a genetic predisposition to hyperinflammation. Colitis and other inflammatory organ pathology associated with granuloma formation are well-known complications in this disease,[47] which were recently linked to autophagic dysfunction[48]. This apparent association of impaired autophagy to the HLH syndrome therefore warrants future investigation. It should be noted that the design of our study did not allow us to exclude a potential contribution of monoallelic mutations or polymorphisms in FHL associated genes to the manifestation of HLH in all patients. Nevertheless, at least seven patients were tested for all known genes associated with FHL and in a number of other patients individual genes have been sequenced without detection of relevant mutations or polymorphisms such as perforin A91V.

An imbalance between virus control and immune activation is thought to be an important determinant for the HLH syndrome associated with viral infections (such as EBV) and in a proportion of patients with FHL. This is supported by evidence from mouse models.[3, 49] In these models, hyperactive T cells are a key factor in disease pathogenesis.[2, 50] It was therefore surprising that, paradoxically, a significant number of patients with T cell deficiencies, including several patients without detectable circulating T cells, developed the HLH syndrome. Notably, at least 3 T cell deficient X-SCID patients also had ≤10/µl NK cells, rendering it unlikely that hyperactivated NK cells replaced T cells as a pathogenic factor in these cases. This is further supported by a significantly lower level of sCD25 in this patient group compared to patients with FHL or I-HLH. These observations suggests that the HLH syndrome can develop despite the severe deficiency of T and NK cells, two main effectors implicated in “primary” HLH. Due to this absence of T and NK cells, the term hemophagocytic *“lympho”* histiocytosis appears inappropriate to describe the “HLH-like” disease in SCID patients. It seems likely that in these patients activation of macrophages and the associated cytokine release proceed independent of lymphocytes. Therefore, the term "hemophagocytic inflammatory syndrome" (HIS) may better describe this condition. Although not addressed in this study, it is possible that such a distinction may also be relevant for some other disease states currently classified as “secondary” HLH.

The current HLH-2004 criteria were insufficient for this discrimination. Neither the number nor the combination of HLH-2004 criteria fulfilled in patients with PID and HIS was different from what is usually observed in “primary” or infection-associated “secondary” HLH. It should be stated that in most PID patients summarized in this study, not all 8 criteria were documented. However, sCD25 is frequently also not determined in many patients with familial HLH and in most centers NK cell cytotoxicity has been replaced by intracellular protein and degranulation studies.[51] The only notable difference between the groups was the relatively low sCD25 levels and high ferritin levels in most patients with T cell deficiencies. This most likely reflects the lack of T cells in SCID and the low T cell counts and/or impaired T cell function in CID patients. Furthermore, the ratio of ferritin to sCD25 heightened these differences and

appears to be a useful measure, as has previously been suggested in lymphoma-associated HLH.[52] Our (admittedly limited) data suggest that a ferritin:sCD25 ratio of $\geq$3 should be viewed as suggestive of SCID/CID in an infant with the HLH syndrome. Because current understanding of HLH pathogenesis suggests that “overactivated” T cells exuberantly recruit innate effectors (macrophages) to drive HLH disease manifestations,[3,53] these findings suggest that uncontrolled viral infections in the context of SCID/CID may circumvent typical HLH pathogenesis. It remains to be shown whether such an alternative pathophysiology may also be relevant for some cases of infection-associated or rheumatologic “secondary” HLH in apparently immunocompetent individuals. In this context it is noteworthy that sCD25 levels were also significantly lower in patients with infection-associated “secondary” HLH than in patients with FHL, though this may simply illustrate the propensity towards T cell ‘overactivation’ in individuals with defective cytotoxic function.

Disease classification should be based on pathophysiological knowledge with the goal to facilitate the best treatment decision for affected patients. The observations of this study further illustrate that the current diagnostic HLH criteria as defined by the HLH-2004 protocol describe a common clinical and biological endpoint resulting from different conditions and pathways of pathogenesis. We think that there are good arguments to reserve the term hemophagocytic “lympho” histiocytosis (HLH) to disease states that are associated with a predominantly T cell mediated immunopathogenesis, including “primary” HLH and some cases of infection-associated “secondary” HLH. In these cases, etoposide-based protocols or T cell directed immune therapy, *e.g.* based on ATG[9] or alemtuzumab,[54] are indicated and can be highly effective. On the contrary, strong immunosuppressive therapy may not always be appropriate and even harmful for other patient groups, especially patients with primary immunodeficiencies other than FHL or XLP. In this study, we propose the provisional term "hemophagocytic inflammatory syndrome" (HIS) to classify these patients, in whom the intensity of immunosuppressive treatment needs to be carefully adapted to the underlying immunodeficiency and ongoing infections.

HIS could be an umbrella term for different diseases currently referred to as “primary” or “secondary” forms of HLH or MAS. In this concept, HLH would be one form of HIS. We propose to maintain the term “hemophagocytic” despite the fact that not all patients fulfill this criterion (41/50 in our study) for the following reasons: 1. The probability to detect hemophagocytosis depends on the time point of the analysis, which is in many cases performed only once. 2. The element of activated phagocytes producing cytokines is likely to be essential for all forms of HIS. 3. Keeping this element will keep the term searchable in the literature and maintain awareness of physicians that this is a serious condition. 4. The term hyperinflammatory alone is poorly discriminative towards other inflammatory conditions. It is important to note that these considerations represent a suggestion and any change in nomenclature should be legitimated by the Histiocyte Society, that is currently engaged in revising the terminology for this group of diseases.

It is an obvious difficulty of current nomenclature that a single clinical scenario (e.g. EBV associated HLH in an infant) could exemplify HLH associated with a defect in lymphocyte cytotoxicity, a T cell driven “secondary” HLH, or a T/NK cell independent HIS in a SCID patient, each with a potentially different pathophysiology and a common phenotypic endpoint. It is thus important to remember that the HLH syndrome itself never represents a complete diagnosis. It will be an important challenge to elaborate diagnostic criteria that can help to separate etiologically different conditions. The ratio of ferritin to sCD25 may represent one of them, but new biomarkers specifically reflecting the individual pathophysiological pathway leading to the inflammatory disease state of the HLH syndrome are needed. It will be of particular interest to evaluate whether markers of immune cell activation in addition to cytokine patterns may more specifically reflect disease pathogenesis.

References

1. Janka GE, Lehmberg K. Hemophagocytic syndromes - an update. Blood Rev. 2014;28(4):135-142.
2. Pachlopnik Schmid J, Cote M, Menager MM, et al. Inherited defects in lymphocyte cytotoxic activity. Immunol Rev. 2010;235(1):10-23.
3. Jordan MB, Hildeman D, Kappler J, Marrack P. An animal model of hemophagocytic lymphohistiocytosis (HLH): CD8+ T cells and interferon gamma are essential for the disorder. Blood. 2004;104(3):735-743.
4. Jordan MB, Allen CE, Weitzman S, Filipovich AH, McClain KL. How I treat hemophagocytic lymphohistiocytosis. Blood. 2011;118(15):4041-4052.
5. Risma K, Jordan MB. Hemophagocytic lymphohistiocytosis: updates and evolving concepts. Curr Opin Pediatr. 2012;24(1):9-15.
6. Castillo L, Carcillo J. Secondary hemophagocytic lymphohistiocytosis and severe sepsis/ systemic inflammatory response syndrome/multiorgan dysfunction syndrome/macrophage activation syndrome share common intermediate phenotypes on a spectrum of inflammation. Pediatr Crit Care Med. 2009;10(3):387-392.
7. Henter JI, Horne A, Arico M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007;48(2):124-131.
8. Henter JI, Samuelsson-Horne A, Arico M, et al. Treatment of hemophagocytic lymphohistiocytosis with HLH-94 immunochemotherapy and bone marrow transplantation. Blood. 2002;100(7):2367-2373.
9. Mahlaoui N, Ouachee-Chardin M, de Saint Basile G, et al. Immunotherapy of familial hemophagocytic lymphohistiocytosis with antithymocyte globulins: a single-center retrospective report of 38 patients. Pediatrics. 2007;120(3):e622-628.
10. Pachlopnik Schmid J, Ho CH, Chretien F, et al. Neutralization of IFNgamma defeats haemophagocytosis in LCMV-infected perforin- and Rab27a-deficient mice. EMBO Mol Med. 2009;1(2):112-124.
11. Faitelson Y, Grunebaum E. Hemophagocytic lymphohistiocytosis and primary immune deficiency disorders. Clin Immunol. 2014;155(1):118-125.
12. Al-Herz W, Bousfiha A, Casanova JL, et al. Primary immunodeficiency diseases: an update on the classification from the international union of immunological societies expert committee for primary immunodeficiency. Front Immunol. 2014;5:162.
13. Lehmberg K, Pink I, Eulenburg C, Beutel K, Maul-Pavicic A, Janka G. Differentiating macrophage activation syndrome in systemic juvenile idiopathic arthritis from other forms of hemophagocytic lymphohistiocytosis. J Pediatr. 2013;162(6):1245-1251.
14. Dvorak CC, Sandford A, Fong A, Cowan MJ, George TI, Lewis DB. Maternal T-cell engraftment associated with severe hemophagocytosis of the bone marrow in untreated X-linked severe combined immunodeficiency. J Pediatr Hematol Oncol. 2008;30(5):396-400.
15. Grunebaum E, Zhang J, Dadi H, Roifman CM. Haemophagocytic lymphohistiocytosis in X-linked severe combined immunodeficiency. Br J Haematol. 2000;108(4):834-837.
16. Patiroglu T, Haluk Akar H, van den Burg M, et al. X-linked severe combined immunodeficiency due to a novel mutation complicated with hemophagocytic lymphohistiocytosis and presented with invagination: A case report. Eur J Microbiol Immunol (Bp). 2014;4(3):174-176.
17. Schmid I, Reiter K, Schuster F, et al. 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;29(6):519-521.
18. Cesaro S, Messina C, Sainati L, Danesino C, Arico M. Del 22Q11.2 and hemophagocytic lymphohistiocytosis: a non-random association. Am J Med Genet A. 2003;116A(2):208-209.
19. Arico M, Bettinelli A, Maccario R, Clementi R, Bossi G, Danesino C. Hemophagocytic lymphohistiocytosis in a patient with deletion of 22q11.2. Am J Med Genet. 1999;87(4):329-330.

20. Pasic S, Micic D, Kuzmanovic M. Epstein-Barr virus-associated haemophagocytic lymphohistiocytosis in Wiskott-Aldrich syndrome. Acta Paediatr. 2003;92(7):859-861.
21. Almagor Y, Revel-Vilk S, Averbuch D, et al. Congenital cytomegalovirus infection and Wiskott-Aldrich syndrome successfully treated with unrelated cord blood transplantation. Pediatr Blood Cancer. 2011;57(4):681-683.
22. Seidel MG. CD27: a new player in the field of common variable immunodeficiency and EBV-associated lymphoproliferative disorder? J Allergy Clin Immunol. 2012;129(4):1175; author reply 1175-6.
23. Salzer E, Daschkey S, Choo S, Gombert et al. Combined immunodeficiency with life-threatening EBV-associated lymphoproliferative disorder in patients lacking functional CD27. Haematologica. 2013;98(3):473-478.
24. Stepensky P, Weintraub M, Yanir A, et al. IL-2-inducible T-cell kinase deficiency: clinical presentation and therapeutic approach. Haematologica. 2011;96(3):472-476.
25. Kashiwagi Y, Kawashima H, Sato S, et al. Virological and immunological characteristics of fatal virus-associated haemophagocytic syndrome (VAHS). Microbiol Immunol. 2007;51(1):53-62.
26. Valentine G, Thomas TA, Nguyen T, Lai YC. Chronic granulomatous disease presenting as hemophagocytic lymphohistiocytosis: a case report. Pediatrics. 2014;134(6):e1727-1730.
27. Araujo A, Pagnier A, Frange P, et al. [Lymphohistiocytic activation syndrome and Burkholderia cepacia complex infection in a child revealing chronic granulomatous disease and chromosomal integration of the HHV-6 genome]. Arch Pediatr. 2011;18(4):416-419.
28. Akagi K, Kawai T, Watanabe N, et al. A case of macrophage activation syndrome developing in a patient with chronic granulomatous disease-associated colitis. J Pediatr Hematol Oncol. 2014;36(3):e169-172.
29. Scheffler-Mendoza SC, Yamazaki-Nakashimada MA, Olaya-Vargas A, et al. Successful stem cell transplantation in a child with chronic granulomatous disease associated with contiguous gene deletion syndrome and complicated by macrophage activation syndrome. Clin Immunol. 2014;154(2):112-115.
30. Alvarez-Cardona A, Rodriguez-Lozano AL, Blancas-Galicia L, Rivas-Larrauri FE, Yamazaki-Nakashimada MA. Intravenous immunoglobulin treatment for macrophage activation syndrome complicating chronic granulomatous disease. J Clin Immunol. 2011;32(2):207-211.
31. van Montfrans JM, Rudd E, van de Corput L, et al. Fatal hemophagocytic lymphohistiocytosis in X-linked chronic granulomatous disease associated with a perforin gene variant. Pediatr Blood Cancer. 2009;52(4):527-529.
32. Martin A, Marques L, Soler-Palacin P, et al. Visceral leishmaniasis associated hemophagocytic syndrome in patients with chronic granulomatous disease. Pediatr Infect Dis J. 2009;28(8):753-754.
33. Hisano M, Sugawara K, Tatsuzawa O, Kitagawa M, Murashima A, Yamaguchi K. Bacteria-associated haemophagocytic syndrome and septic pulmonary embolism caused by Burkholderia cepacia complex in a woman with chronic granulomatous disease. J Med Microbiol. 2007;56(Pt 5):702-705.
34. Sirinavin S, Techasaensiri C, Pakakasama S, Vorachit M, Pornkul R, Wacharasin R. Hemophagocytic syndrome and Burkholderia cepacia splenic microabscesses in a child with chronic granulomatous disease. Pediatr Infect Dis J. 2004;23(9):882-884.
35. Parekh C, Hofstra T, Church JA, Coates TD. Hemophagocytic lymphohistiocytosis in children with chronic granulomatous disease. Pediatr Blood Cancer. 2011;56(3):460-2.
36. Schultz KA, Neglia JP, Smith AR, Ochs HD, Torgerson TR, Kumar A. Familial hemophagocytic lymphohistiocytosis in two brothers with X-linked agammaglobulinemia. Pediatr Blood Cancer. 2008;51(2):293-295.
37. Kuijpers TW, Baars PA, vaan de Kerk DJ, et al. Common variable immunodeficiency and hemophagocytic features associated with a FAS gene mutation. J Allergy Clin Immunol. 2011;127(6):1411-1414 e2.

38. Rudman Spergel A, Walkovich K, Price S, et al. Autoimmune lymphoproliferative syndrome misdiagnosed as hemophagocytic lymphohistiocytosis. Pediatrics. 2013;132(5):e1440-1444.
39. Pachlopnik Schmid JM, Junge SA, Hossle JP, et al. Transient hemophagocytosis with deficient cellular cytotoxicity, monoclonal immunoglobulin M gammopathy, increased T-cell numbers, and hypomorphic NEMO mutation. Pediatrics. 2006;117(5):e1049-1056.
40. Horneff G, Rhouma A, Weber C, Lohse P. Macrophage activation syndrome as the initial manifestation of tumour necrosis factor receptor 1-associated periodic syndrome (TRAPS). Clin Exp Rheumatol. 2013;31(3 Suppl 77):99-102.
41. Rossi-Semerano L, Hermeziu B, Fabre M, Kone-Paut I. Macrophage activation syndrome revealing familial Mediterranean fever. Arthritis Care Res. 2011;63(5):780-783.
42. Uslu N, Demir H, Balta G, et al. Hemophagocytic syndrome in a child with severe Crohn's disease and familial Mediterranean fever. J Crohns Colitis. 2010;4(3):341-344.
43. Canna SW, de Jesus AA, Gouni S, et al. An activating NLRC4 inflammasome mutation causes autoinflammation with recurrent macrophage activation syndrome. Nat Genet. 2014;46(10):1140-1146.
44. Felgentreff K, Perez-Becker R, Speckmann C, et al. Clinical and immunological manifestations of patients with atypical severe combined immunodeficiency. Clin Immunol. 2011;141(1):73-82.
45. Ghosh S, Bienemann K, Boztug K, Borkhardt A. Interleukin-2-inducible T-cell kinase (ITK) deficiency - clinical and molecular aspects. J Clin Immunol. 2014;34(8):892-899.
46. van Montfrans JM, Hoepelman AI, Otto S, et al. CD27 deficiency is associated with combined immunodeficiency and persistent symptomatic EBV viremia. J Allergy Clin Immunol. 2012;129(3):787-793 e6.
47. Magnani A, Brosselin P, Beaute J, et al. Inflammatory manifestations in a single-center cohort of patients with chronic granulomatous disease. J Allergy Clin Immunol. 2014;134(3):655-662 e8.
48. de Luca A, Smeekens SP, Casagrande A, et al. IL-1 receptor blockade restores autophagy and reduces inflammation in chronic granulomatous disease in mice and in humans. Proc Natl Acad Sci U S A. 2014;111(9):3526-3531.
49. Jessen B, Kogl T, Sepulveda FE, de Saint Basile G, Aichele P, Ehl S. Graded defects in cytotoxicity determine severity of hemophagocytic lymphohistiocytosis in humans and mice. Front Immunol. 2013;4:448.
50. Lykens JE, Terrell CE, Zoller EE, Risma K, Jordan MB. Perforin is a critical physiologic regulator of T-cell activation. Blood. 2011;118(3):618-626.
51. Bryceson YT, Pende D, Maul-Pavicic A, et al. A prospective evaluation of degranulation assays in the rapid diagnosis of familial hemophagocytic syndromes. Blood. 2012;119(12):2754-2763.
52. Tabata C, Tabata R. Possible prediction of underlying lymphoma by high sIL-2R/ferritin ratio in hemophagocytic syndrome. Ann Hematol 2012;91:63–71.
53. Zoller EE, Lykens JE, Terrell CE, et al. Hemophagocytosis causes a consumptive anemia of inflammation. J Exp Med. 2011;208(6):1203-1214.
54. Marsh RA, Allen CE, McClain KL, et al. Salvage therapy of refractory hemophagocytic lymphohistiocytosis with alemtuzumab. Pediatr Blood Cancer. 2013;60(1):101-109.

Tables & Figure legends

Patient no.	PID *(affected gene)*	Age at HLH [y]	HLH at/before PID diagnosis	Associated infection	HLH directed therapy	Out-come	Ref.
SCID							
1	SCID (*ILR2G*)	0.13	+	Rhinovirus, Enterobacter	IVIG, CsA	√	[14]
2	SCID (*ILR2G*)	0.17	+	gram negative sepsis	steroids, eto	†	[15]
3	SCID *(ILR2G*)	0.3	+	*M. tuberculosis*	IVIG	√	
4	SCID (*ILR2G*)	0.3	+	CMV	steroids, eto	√	
5	SCID (*ILR2G*)	0.3	-	*P. aeruginosa*	IVIG	†	[16]
6	SCID (*RAG-1*)	1	-	Adenovirus	-	†	
7	SCID (*RAG-1*)	1.5	+	Adenovirus	steroids	†	
8	SCID (*IL7RA*)	0.21	+	EBV	HLH 94	†	
9	SCID (*CD3E*)	0.5	+	Adenovirus	-	†	
10	SCID (T-, B-, NK+)	0.3	?	CMV	IVIG	†	
11	SCID (T-, B+, NK+)	0.42	+	EBV	steroids, eto	†	[17]
12	SCID (undefined)	0.4	+	PJ, CMV	-	√	
CID							
13	CID (clinical Omenn)*	1.3	-	*St. maltophilia, Alternaria*	-	†	
14	CID (*RAG-1*)*	1	+	-	-	√	
15	22q11	0.3	-	-	-	?	
16	22q11	1.1	-	-	HLH-1994	†	[18]
17	22q11	1.5	-	EBV	IVIG, steroids	√	
18	22q11	5.6	-	EBV	HLH-1994	†	[19]
19	WAS	0.3	-	EBV	HLH-1994	†	[20]
20	WAS	0.6	+	CMV	IVIG, steroids	√	[21]
21	WAS	1.3	-	CMV	IVIG, steroids	†	
22	WAS	1.5	+	CMV	-	√	
23	Ataxia telangiectasia	17.3	-	EBV, parvovirus	steroids, CsA	†	
24	DKC1	4	+	EBV	-	√	
25	CD 27	1.5	+	EBV	IVIG, steroids, ritux,	√	[22, 23]
26	CD 27	1.5	+	EBV	ritux, HLH-2004	√	[22, 23]
27	ITK	5	+	EBV	steroids, ritux, eto	†	[24]
28	CID (predominantely T-)	0,17	+	CMV	-	†	[25]
29	CID (undefined)	0.5	+	Noro/Rotavirus	IVIG	√	
30	CID (undefined)	4.4	-	-	HLH-1994/2004	√	
CGD							
31	CGD (*p91*)	0.17	+	*C. lusitaniae*, MRSA	IVIG, steroids	√	
32	CGD (*p91*)	0.17	+	*C. lusitaniae*	IVIG, steroids, CsA	√	[26]
33	CGD (*p91*)	0.9	+	*B. cepacia,* HHV-6	steroids	√	[27]
34	CGD (*p91*)	2	-	-	steroids	√	[28]
35	CGD *(p91)*	2	+	*P. spp., Salmonella spp.*	steroids, CsA	√	[29]
36	CGD (*p91*)	3	-	*B. cepacia*	IVIG, steroids	√	[30]
37	CGD (*p91*)	3.5	-	-	steroids	√	
38	CGD (*p91*)	3.5	+	*B. cepacia, St. maltophilia*	HLH-2004	†	[31]
39	CGD (*p91*)	15	-	Leishmania spp.	-	√	[32]
40	CGD (*p91*)	18	-	Leishmania spp.	-	†	[32]
41	CGD (*p91*)	24	-	Leishmania spp.	IVIG, steroids	√	
42	CGD (*p91*)	26	-	Leishmania spp.	IVIG, steroids	√	
43	CGD (*p91*)	34	-	Leishmania spp.	IVIG, steroids	√	
44	CGD (*p47*)	2.5	+	*B. cepacia,* EBV	steroids, CsA	√	
45	CGD (*p47*)	9	?	Leishmania spp.	IVIG	√	[32]
46	CGD (*p22*)	0.8	+	*C. parapsilopsis*	steroids	√	
47	CGD (*p22*)	29	-	*B. cepacia*	steroids, CsA	√	[33]
48	CGD (DHR)	1.4	+	*B. cepacia*	IVIG	√	[34]
49	CGD (DHR)	3.7	+	*B.cepacia*	IVIG, steroids	√	
50	CGD (clinical)	6	-	*E. cloacae, S. epidermidis*	IVIG, steroids	√	[35]
51	CGD (clinical)	7	-	Aspergillus spp.	IVIG, steroids	√	[35]
52	CGD (clinical)	12	-	-	IVIG, steroids	√	[35]
others							
53	XLA (*BTK*)	0.7	+	Adenovirus	IVIG, HLH-2004	†	[36]
54	XLA (*BTK*)	3	+	Adenovirus	IVIG	√	[36]
55	ALPS *(FAS)*	3	+	-	IVIG, steroids	√	[37]
56	ALPS *(FAS)*	6	+	-	HLH-2004	√	[38]
57	Cyclic neutropenia	19	-	EBV	IVIG, steroids	?	
58	NEMO	0.2	+	*K. pneumoniae*	IVIG	√	[39]
59	STAT1 (GOF)	0.15	+	-	steroids	†	
60	TRAPS	11	+	-	HLH-2004	√	[40]
61	FMF	4	+	-	steroids, CsA	√	[41]
62	FMF	11	-	*A. baumanii*	IVIG, steroids, CsA	†	[42]
63	*NLRC4*	7	+	-	steroids	√	[43]

Table 1. Cohort of patients with HLH syndrome in the context of primary immunodeficiencies. Abbreviations:; √ = alive; † = deceased; n.a. = not available. A. = *Acinetobacter*, ALPS = autoimmune lymphoproliferative syndrome, B. = *Burkholderia*, C. = *Candida*, CGD = Chronic granulomatous disease, CID = combined immunodeficiency, GOF = gain of function, CMV = cytomegalovirus, CsA = cyclosporin A, DKC = Dyskeratosis congenita, E.= *Enterobacter*, EBV = Epstein Barr virus, FMF = Familial Mediterranean Fever, IVIG = intravenous immunoglobulins, M. = *Mycobacterium*, MRSA = methicillin resistant *Staphylococcus aureus*, P. = *Pseudomonas*, PJ = *Pneumocystis jirovecii*, ritux = rituximab, S. = *Staphylococcus*, SCT = stem cell transplantation, spp. = Species, St. = *Stenotrophomonas*, TRAPS = TNF-1 receptor associated periodic syndrome, eto = etoposide, WAS = Wiskott Aldrich syndrome, XLA = X-linked agammaglobulinemia. * = these patients did not qualify as SCID as they had significant residual autologous T, B, and NK cells

Patient no.	CD3 T cells/µl	CD4+ T cells/µl	CD8+ T cells/µl	NK cells/µl	B cells/µl	materno-fetal transfusion
1	30	0	20	0	0	+
2	18	10	8	32	85	n.a.
3	0	0	0	9	1178	-
4	30	n.a.	n.a.	95	0	+
5	18	16	0	10	990	n.a.
6	14	9	5	59	5	n.a.
7	84	26	57	2143	466	-
8	20	<10	<20	n.a.	< 20	n.a.
9	0	0	0	45	959	n.a.
10	4	2	1	146	5	n.a.
11	0	0	0	n.a.*	1920	n.a.
12	16	16	<1	296	1574	n.a.

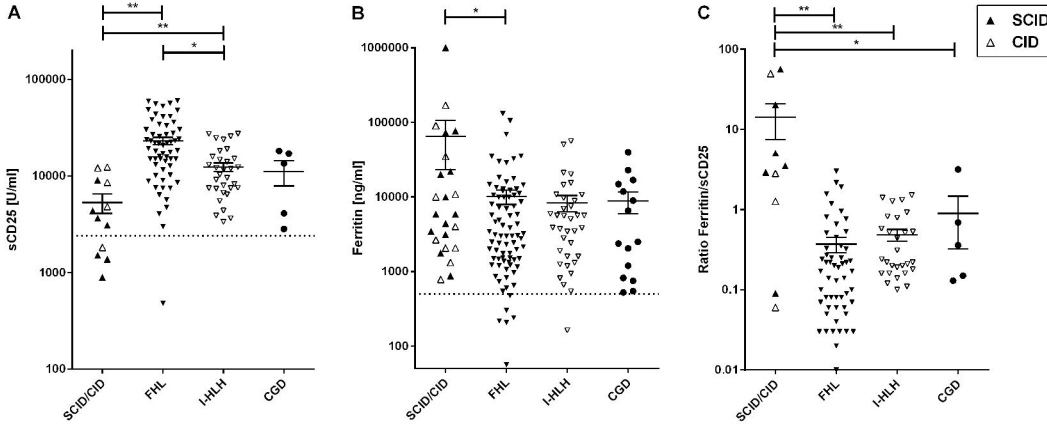
Table 2. Lymphocyte subsets in patients with SCID and hemophagocytic inflammatory syndrome. n.a. = no data available. *: According to [17] NK+.

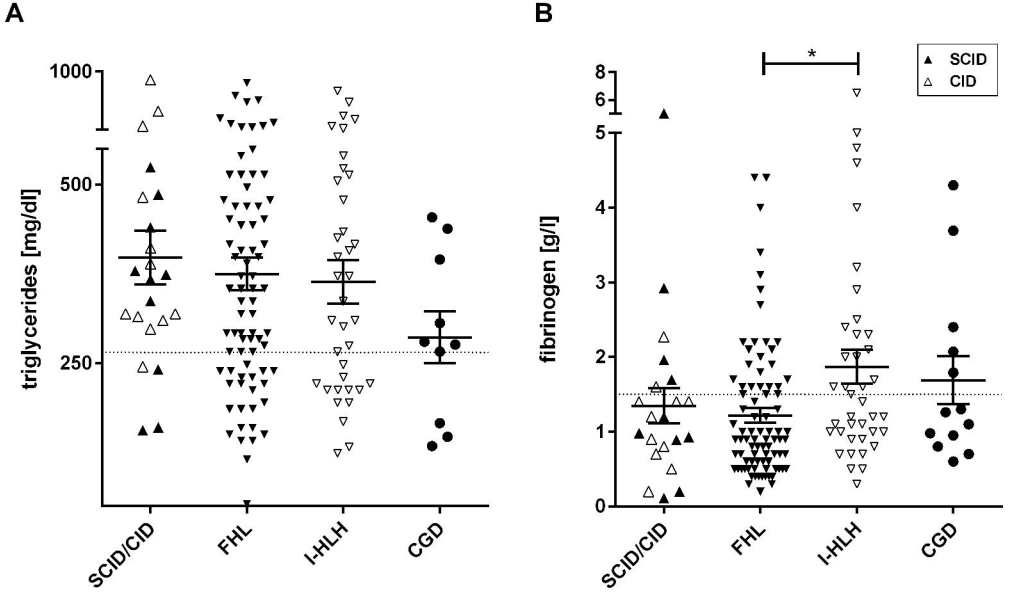
Patient no.	Fever	Splenomegaly	Cytopenia 2/3 cell lines	Triglycerides [mg/dl]	Fibrinogen [g/l]	Hemophagocytosis	Ferritin [ng/ml]	sCD25 [U/ml]	NK cytotoxicity	HLH criteria
SCID										
1	+	-	+	n.a.	n.a.	+	**872**	**9016**	n.a.	5/5
2	+	+	+	**374**	**0.2**	+	**1774**	n.a.	n.a.	6/6
3	+	+	+	**486**	n.a.	+	**5866**	n.a.	n.a.	6/6
4	+	+	-	**337**	1.7	n.a.	**22000**	**4349**	**reduced**	6/6
5	+	+	-	**524**	**0.11**	+	**9973**	n.a.	n.a.	5/6
6	+	+	+	160	**0.89**	+	**77000**	1365	n.a.	6/7
7	+	-	+	**440**	**0.93**	+	**3133**	890	normal	5/7
8	+	+	+	156	**1.2**	+	**3474**	n.a.	n.a.	6/6
9	+	-	+	n.a.	**0.98**	+	**>1.000.000**	**3078**	na	6/7
10	+	+	+	**379**	2.92	+	**4400**	1509	n.a.	6/7
11	+	+	+	241	5.02	+	**5866**	n.a.	n.a.	5/6
12	+	+	-	**367**	1.96	n.a.	**73100**	**3635**	n.a.	5/6
CID										
13	+	+	+	**389**	**1.4**	n.a.	**2066**	n.a.	n.a.	5/5
14	+	+	+	n.a.	**<1.5**	n.a.	**>500**	n.a.	n.a.	5/5
15	+	+	+	**>265**	n.a.	n.a.	n.a.	**4803**	n.a.	5/5
16	+	+	+	**693**	**0.8**	+	**10854**	**8540**	**reduced**	8/8
17	+	+	+	**575**	**0.5**	+	**4000**	n.a.	n.a.	6/6
18	+	+	+	**>265**	**<1.5**	+	**>500**	n.a.	n.a.	6/6
19	+	+	+	**315**	**1.4**	+	**169195**	n.a.	**reduced**	7/7
20	+	+	+	245	2.27	-	**785**	**12080**	n.a.	5/7
21	+	-	+	**310**	**0.9**	+	**2650**	n.a.	n.a.	5/6
22	+	+	+	**938**	**1.2**	-	**1320**	n.a.	normal	5/7
23	+	+	+	**319**	**1.4**	+	**35000**	**12400**	n.a.	7/7
24	+	+	+	**319**	1.6	-	**2058**	n.a.	**reduced**	5/7
25	+	+	+	**>265**	**<1.5**	-	**>500**	n.a.	**reduced**	6/7
26	+	n.a.	+	**n.a.**	**<1.5**	n.a.	**>500**	n.a.	**reduced**	5/5
27	+	+	+	**298**	**0.2**	+	**>7500**	n.a.	n.a.	6/6
28	+	+	+	**n.a.**	**n.a.**	+	**>10000**	n.a.	**n.a.**	5/5
29	+	+	-	**482**	**0.7**	+	**90000**	1815	**absent**	6/8
30	+	+	+	**411**	>1.5	+	**>20000**	n.a.	**reduced**	7/7
CGD										
31	+	+	+	**276**	**1.1**	n.a.	**2039**	**13483**	normal	6/7
32	+	+	+	n.a.	**0.8**	+	**11783**	**17035**	n.a.	7/7
33	+	+	+	n.a.	2.4	+	**16800**	n.a.	n.a.	5/6
34	+	+	+	n.a.	n.a.	n.a.	**14838**	n.a.	n.a.	5/5
35	+	+	+	**>265**	n.a.	+	**>500**	n.a.	n.a.	6/6
36	+	+	+	**395**	1.79	n.a.	**750**	n.a.	n.a.	5/5
37	+	+	+	**>265**	**1.3**	-	**2500**	n.a.	n.a.	5/6
38	+	+	+	**280**	**0.7**	-	**6539**	**18115**	n.a.	6/7
39	+	+	+	**>265**	**<1.5**	+	n.a.	n.a.	n.a.	5/5
40	+	+	+	**>265**	**<1.5**	+	**>500**	n.a.	n.a.	6/6
41	+	+	+	**454**	**0.98**	+	**1192**	n.a.	n.a.	6/6
42	+	+	+	n.a.	**0.95**	+	**39734**	n.a.	n.a.	6/6
43	+	+	+	n.a.	n.a.	+	**2378**	n.a.	n.a.	5/5
44	+	+	+	**>265**	n.a.	n.a.	**>500**	n.a.	n.a.	5/5
45	+	+	+	**>265**	n.a.	+	**>500**	n.a.	n.a.	6/6
46	+	+	+	**306**	2.07	-	**546**	**4097**	**reduced**	7/8
47	+	+	-	n.a.	4.3	+	**820**	**>2400**	n.a.	5/6
48	+	+	+	**266**	n.a.	+	n.a.	n.a.	n.a.	5/5
49	+	+	+	166	**0.6**	n.a.	**22919**	n.a.	n.a.	5/5
50	+	+	+	134	**<0.5**	+	**523**	n.a.	n.a.	6/6
51	+	+	+	**438**	**1.26**	+	n.a.	n.a.	n.a.	5/5
52	+	+	+	147	3.69	+	**8930**	**2816**	n.a.	6/7
others										
53	+	-	+	138	**0.87**	+	**54399**	n.a.	**absent**	6/7
54	+	-	+	**287**	**1.25**	+	**19886**	**>2400**	**reduced**	7/8
55	+	+	+	**350**	n.a.	-	**2760**	**15570**	n.a.	6/6
56	-	+	+	**292**	n.a.	-	**809**	**18651**	normal	5/7
57	+	+	+	**>265**	**<1.5**	n.a.	**>500**	**>7500**	n.a.	6/6
58	+	+	+	n.a.	**1**	+	**27750**	n.a.	**reduced**	7/7
59	+	+	+	**>265**	**<1.5**	+	**>500**	n.a.	n.a.	6/6
60	+	+	n.a.	n.a.	n.a.	+	**8030**	**>2400**	normal	5/6
61	+	+	+	n.a.	**1.3**	+	n.a.	n.a.	normal	5/6
62	+	+	+	261	n.a.	+	**672**	n.a.	n.a.	5/6
63	+	+	+	**>265**	n.a.	n.a.	**>500**	n.a.	normal	5/6

Table 3. HLH diagnostic criteria in patients with PID and hemophagocytic inflammatory syndrome. n.a. = no data available. Parameters fulfilling the HLH diagnostic criteria are indicated in bold. The last column indicates the number of abnormal criteria relative to the number of criteria investigated/reported.

Figure 1. A: Serum sCD25 levels in patients with SCID/CID, FHL, I-HLH (infection triggered "secondary" HLH), and CGD. **B:** Serum ferritin levels in SCID/CID, FHL, I-HLH, and CGD patients. **C:** Ratio of Ferritin/sCD25 in SCID/CID, FHL, I-HLH, and CGD patients. * = $p<0.05$. ** = $p<0.01$. The dotted line indicates the cut-off value according to the HLH diagnostic criteria. The bars indicate means ± standard deviation.

Figure 2. A: Serum triglyceride levels in SCID/CID, FHL, I-HLH, and CGD patients. **B:** Serum fibrinogen levels in SCID/CID, FHL, I-HLH, and CGD patients. * = $p<0.05$. The dotted line indicates the cut-off value according to the HLH diagnostic criteria. The bars indicate means ± standard deviation.


A
B
C
SCID
CID
sCD25 [U/ml]
Ferritin [ng/ml]
Ratio Ferritin/sCD25
100000
10000
1000
100
1000000
10
1
0.1
0.01
**
**
*
*
*
SCID/CID
FHL
I-HLH
CGD


A
1000
500
250
triglycerides [mg/dl]
SCID/CID
FHL
I-HLH
CGD
B
8
6
5
4
3
2
1
0
fibrinogen [g/l]
*
SCID
CID
SCID/CID
FHL
I-HLH
CGD