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OUTCOME OF ADULT PATIENTS WITH HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS: A RETROSPECTIVE ANALYSIS

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Zusammenfassung:

Die hämophagozytische Lymphohistiozytose (HLH) ist ein seltenes, doch häufig schwerwiegend verlaufendes hyperinflammatorisches Syndrom. Beim Erwachsenen tritt die HLH typischerweise im Rahmen von Malignomen, Infektionen, Autoimmunerkrankungen oder unter immunsuppressiven Therapien auf. Trotz einer zunehmenden Aufmerksamkeit der HLH beim Erwachsenen, basiert der Großteil der angewandten diagnostischen und therapeutischen Leitlinien nach wie vor auf pädiatrischen Studien. Um mögliche Risikofaktoren sowie das Outcome von erwachsenen Patienten mit HLH zu analysieren, wurde eine multizentrische retrospektive Studie unter besonderer Berücksichtigung des klinischen Verlaufs und der angewandten diagnostischen und therapeutischen Maßnahmen durchgeführt. Insgesamt konnten 62 erwachsene Patienten (≥ 18 Jahre) aus vier Münchner Kliniken in die Analyse eingeschlossen werden. Der Großteil der 62 Patienten war männlich (40/62, 65%) und der Altersmedian lag bei 53,5 Jahren (Spannweite, 19-81 Jahre). Alle Patienten wurden anhand der wahrscheinlichsten Ursache ihrer HLH in vier ätiologische Gruppen unterteilt: bei jeweils 22 Individuen trat die HLH im Zusammenhang mit Malignomen (35%) und Infektionen (35%) auf. Bei 10 Patienten (16%) lag eine Autoimmunerkrankung vor und in den verbleibenden 8 Personen (13%) konnte kein spezifischer Auslöser identifiziert werden. In Hinblick auf die diagnostischen Kriterien wiesen alle Patienten erhöhte Ferritinwerte > 500 µg/L auf. Die Gesamtletalität bei einem medianen Nachbeobachtungszeitraum von 288 Tagen lag bei 52% (32/62). Die Gesamtüberlebenswahrscheinlichkeit innerhalb der vier ätiologischen Subgruppen unterschied sich signifikant (p = 0,004, Log-Rank-Test). Die niedrigste Überlebenswahrscheinlichkeit zeigte sich hierbei in der mit Malignomen assoziierten Patientengruppe, in der die Überlebenswahrscheinlichkeit nach einem Jahr bei lediglich 22% lag. Dahingegen lag die Einjahresüberlebenswahrscheinlichkeit der Subpopulation mit infektassoziierter HLH bei 62%, die der autoimmunassoziierten HLH bei 90%, und die der idiopathischen HLH bei 46%. Im univariaten Cox-Regressions-Modell waren folgende Parameter mit einer signifikant (p < 0,1) erhöhten Hazard Ratio in Bezug auf das Gesamtüberleben assoziiert: höheres Alter, Vorliegen eines Malignoms, erhöhte Serumspiegel von Kreatinin, LDH, ASAT, INR, sCD25, sowie niedrigere Thrombozytenzahlen und Albuminwerte bei Therapieeinleitung. Im multivariaten Cox-Regressionsmodell waren einzig erhöhte sCD25 Werte bei Therapieeinleitung mit einem signifikant schlechteren Gesamtüberleben assoziiert (p = 0,005). Die Ergebnisse der Studie legen nahe, dass sCD25 einen möglichen prognostischen Faktor bei erwachsenen Patienten mit HLH darstellt, der im

klinischen Alltag auch zur Entscheidungsfindung bezüglich der Wahl der geeigneten Therapie Anwendung finden könnte. [1, 2]

Abstract:

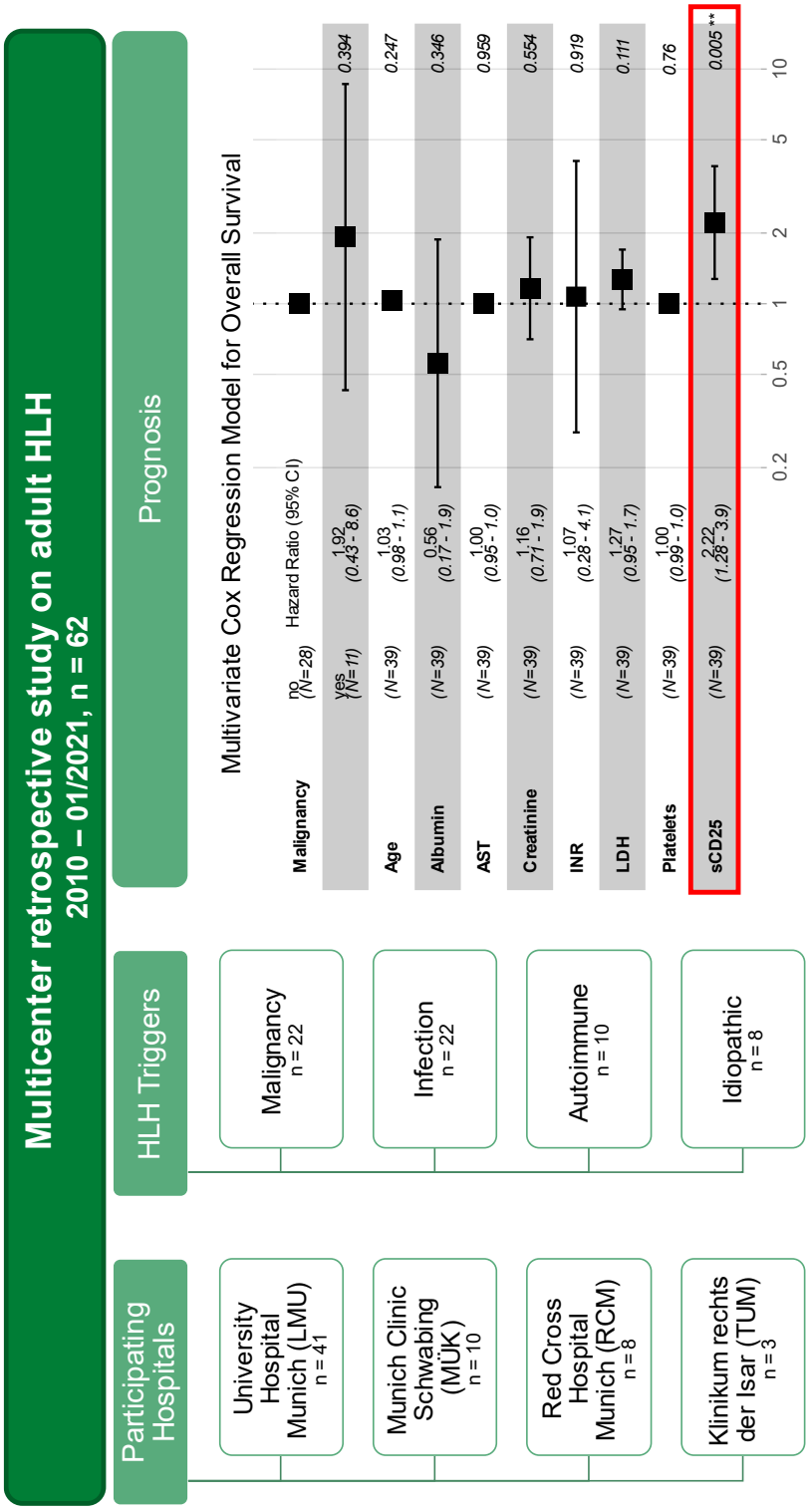
Hemophagocytic lymphohistiocytosis (HLH) is a rare but often fatal hyperinflammatory syndrome. In adults, HLH usually manifests itself along with infections, malignancies, autoimmune diseases, and immunosuppressive treatment. The last years have been marked by an increasing awareness that is also reflected in a rising number of publications addressing HLH in adults. However, the most common diagnostic and therapeutic criteria are still based on the Histiocyte Society's pediatric HLH-94 and HLH-2004 trials. This multicenter retrospective study in Munich was conducted in order to analyze risk factors and outcome of adult HLH patients. [1, 2]

Of 62 patients meeting the inclusion criteria, 40 (65%) were male and the median age at diagnosis was 53.5 years (range, 19-81 years). All patients were assigned to four etiologic subgroups based on the most likely trigger of their HLH episode: 22 (35%) had an underlying malignancy, 22 (35%) an infection, 10 (16%) an autoimmune disease, and no specific trigger could be identified in the remaining 8 individuals (13%). All patients presented elevated ferritin values > 500 µg/L. Thirty-two patients (52%) died at a medium follow-up time of 288 days. [1, 2]

There was a significant difference in the survival probability of the four etiologic subgroups ($p = 0.004$, log-rank test). Patients with HLH triggered by malignancy showed the poorest clinical outcome with a one-year survival probability of 22%. The one-year survival probability of the other subgroups reached 62% for infection associated HLH, 90% for autoimmune associated HLH, and 46% for idiopathic HLH. Parameters, which were significantly ($p < 0.1$) associated with worse overall survival in the univariate Cox regression model were: older age, malignant trigger, elevated serum levels of AST, creatinine, INR, LDH, sCD25 as well as a lowered platelet count and albumin level at treatment initiation. In multivariate analysis sCD25 was the only significant prognostic factor ($p = 0.005$). [1, 2]

The results of this study suggest that sCD25 might be a useful adverse prognostic parameter for adult HLH patients that could help to stratify therapeutic procedures. [1, 2]

Graphical Abstract:



Wimmer et al., *sCD25 as an independent adverse prognostic factor in adult patients with HLH: results of a multicenter retrospective study* © 2023 by Elsevier [2], permission of the publisher as author of the article not required.

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Abbreviations

^{18}F-FDG	2-deoxy-2-[18F]fluoro-D-glucose
alloSCT	allogeneic stem cell transplantation
ALT	alanine transaminase
AML	acute myeloid leukemia
AOSD	adult-onset Still's disease
AP	alkaline phosphatase
aPPT	activated partial thromboplastin time
AST	aspartate transaminase
ATG	anti-thymocyte globulin
AUC	area under the curve
BMT	bone marrow transplantation
CART	chimeric antigen receptor T-cells
CHS	Chédiak-Higashi syndrome
CI	confidence interval
CLIA	chemiluminescent immunoassay
CMV	cytomegalovirus
CNS	central nervous system
CRP	c-reactive protein
CSA	cyclosporine A
CSF	cerebrospinal fluid
CT	computed tomography
CTL	cytotoxic T lymphocyte
DEP	doxorubicin, etoposide, methylprednisolone
DIC	disseminated intravascular coagulopathy
DLBCL	diffuse large B-cell lymphoma

EBV	Epstein–Barr virus
ELISA	enzyme-linked immunosorbent assay
FHL	familial hemophagocytic lymphohistiocytosis
GGT	gamma-glutamyltransferase
GS-2	Griscelli syndrome type 2
HHV	human herpesvirus
HIV	human immunodeficiency virus
HLH	hemophagocytic lymphohistiocytosis
HPS2	Hermansky-Pudlak syndrome type 2
HSV	herpes simplex virus
ICU	intensive care unit
IFNγ	interferon gamma
IL	interleukin
INR	international normalized ratio
i.v.	intravenous
IVIGs	intravenous immune globulins
JAK	Janus kinase
LDH	lactate dehydrogenase
MAS	macrophage activation syndrome
MDS	myelodysplastic syndrome
NHL	non-Hodgkin lymphoma
NK cell	natural killer cell
PCR	polymerase chain reaction
PCT	procalcitonin
PET	positron-emission tomography
PTCL-NOS	peripheral T-cell lymphoma not otherwise specified
sCD25	alpha chain of the soluble interleukin-2 receptor

SCT	stem cell transplantation
sJIA	systemic juvenile idiopathic arthritis
SLE	systemic lupus erythematosus
SUV	standardized uptake value
TNFα	tumor necrosis factor α
WES	whole exome sequencing
XIAP	X-linked inhibitor of apoptosis
XLP	X-linked lymphoproliferative disease

1. Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a rare but often fatal hyperinflammatory syndrome due to an overly but dysfunctional activation of the immune system [3]. Manifestations of HLH can appear at any age and the syndrome is traditionally divided into a primary (genetic) and secondary (acquired) form [4]. The primary form predominantly appears due to autosomal recessive mutations in genes associated with the granule-dependent immune response of cytotoxic lymphocytes such as cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells [3]. With regard to its hereditary background, primary HLH usually manifests itself within the first two years of life [5] and the most relevant recessive inherited entities are subsumed in the familial hemophagocytic lymphohistiocytosis (FHL) syndromes 1-5 [3]. In the secondary form, HLH appears in the context of an immune disbalance along with severe infections, malignancies, autoimmune diseases, and immunosuppressive treatment. Secondary/acquired HLH is the predominant form in older children and adults [3]. However, given the increased identification of heterozygous and hypomorphic mutations in patients with acquired HLH, the differentiation between the two entities has become progressively blurred within the last decades [6, 7].

Because of the heterogenous clinical manifestations and severity of disease, difficulties arise with regard to diagnosis and therapy [5]. Despite all progresses made in establishing diagnostic criteria, treatment protocols, and an increased understanding of the pathophysiology, overall mortality in adults still surpasses at least 40% [8]. Ramos-Casals et al., who conducted one of the largest literature research projects on HLH in adults, referred to it as “one of the most critical clinical disorders in adults” [8].

In order to improve the overall understanding and clinical management of adult HLH patients, we conducted a retrospective study with special attention to epidemiology, etiology, diagnostic and therapeutic measurements, clinical outcome, and prognosis. Therefore, we retrospectively analyzed all adult HLH patients, who were treated in the participating departments of four Munich hospitals. With four big hematologic/oncologic clinics cooperating, it should have been possible to include the majority of adult HLH patients who were treated in the metropolitan area of Munich within the study period from 01/2010 to 01/2021.

1.1 Historical milestones

The first case series on HLH, then referred to as *Histiocytic Medullary Reticulosis*, was published by Scott and Robb-Smith in 1939 and described solely deceased adult patients who shared a collective clinical constellation of fever, lymphadenopathy, hepatosplenomegaly, and postmortem findings of generalized erythrophagocytosis [9].

The majority of subsequent studies was based on pediatric populations. In 1952, Farquhar and Claireaux introduced the term *Familial haemophagocytic reticulosis* in a case description of two siblings who died at the age of nine weeks after developing a fatal symptom complex consisting of fever, hepatosplenomegaly, pancytopenia, and signs of hemophagocytosis in the reticuloendothelial system [10].

In 1979, Risdall et al. introduced the term *virus-associated hemophagocytic syndrome* (VAHS) by describing hemophagocytosis figures in benign histiocytes of 19 patients in association with viral infections and immunosuppression [11].

In 1994, the Histiocyte Society proposed its first protocols on diagnosis and treatment alongside an international prospective study in the pediatric population [12]: their dexamethasone and etoposide containing chemoimmunotherapeutic regimen managed to improve long time survival of affected children from < 5% to > 50% [12-14]. The diagnostic criteria of the revised HLH-2004 protocol were published in 2007 and remain the most widely used to date [15].

A major step forward in the understanding of the pathophysiology was made in 1999, when the first gene associated with the development of HLH in an autosomal-recessive pattern was identified to be the perforin encoding *PRF1* [16].

Once the pivotal role that cytokines play in the pathogenesis of HLH was discovered, the expression *hypercytokinemia* was established in order to describe the pathophysiologic processes in FHL patients [17, 18].

In the last years, with regard to the progresses made in genetic testing – first and foremost the increased availability of whole exome sequencing (WES) – more relevant genes associated with the development of HLH were identified. Thus, through the detection of monoallelic mutations in adults with secondary HLH [7], a threshold model of continuous risk of developing HLH, including both endogen (e.g., genetic) and exogen (environmental) factors, has been proposed (Figure 4) [6, 7, 19, 20].

For a better understanding and clearer representation of the etiology and pathophysiology, both the primary and secondary form are described separately in the following text in accordance with the *revised classification of histiocytoses and neoplasms of the macrophage-dendritic cell lineages* [21].

1.2 Primary HLH

HLH due to inborn errors of immunity, is usually referred to as primary or genetic HLH [22]. A further differentiation is made between the autosomal recessive inherited FHL syndromes and HLH associated with other hereditary immune deficiency syndromes. Whereas HLH is the only disease manifestation in FHL syndromes, it represents a common but not mandatory complication in the other immune deficiency syndromes [3]. Despite the inverse correlation between manifestation of hereditary HLH and increasing age at the onset of disease [23], manifestations of primary HLH have been described to occur from any time between in utero [24] until the 6th decade of life [25]. Thus, the differentiation between primary and secondary HLH solely based on patient's age at onset of disease, even though raising suspicion into one direction or another, is ultimately not possible. Concerning the incidence of primary HLH in children, two subsequent studies in Sweden, regarding each the time period from 1971 to 1986 and 1987 to 2006, described a consistent incidence of 1.2 per 1,000,000 children per year, corresponding to approximately 1:50,000 live births [26, 27].

1.2.1 Familial hemophagocytic lymphohistiocytosis (FHL)

The first gene to be identified with primary HLH due to biallelic mutations, was the perforin encoding *PRF1* in 1999 [16]. Perforin plays a central role in the granule-dependent toxicity of CTLs and NK cells [28]. Further autosomal recessive mutations in genes associated with the cytotoxic granule pathway [29], were detected in the subsequent decade and form the FHL syndromes 2-5: besides perforin (*PRF1*; FHL2) [16], mutations in the genes encoding for Munc13-4 (*UNC13D*; FHL3) [30], Syntaxin 11 (*STX11*; FHL4) [31], and Munc18-2 (*STXBP2*; FHL5) [32, 33] have been discovered. Note that the protein associated with FHL1 has not been identified yet [22], but its gene locus on chromosome 9 (9q21) is known since 1999 [34].

In a retrospective Italian study including 186 patients with suspected primary HLH, the two most frequent mutations affected *PRF1* (FHL2) and *UNC13D* (FHL3) with a

prevalence of 37 and 33%, respectively [20]. Recently, compound heterozygous mutations in the *RHOG* gene, encoding for the small GTPase RHoG, were identified in a four months old boy with confirmed HLH diagnosis via the use of WES [35]. Through the application of model cell lines, the authors discovered the pivotal role that RHoG is playing in the exocytotic pathway of lymphocytes. Therefore, they proposed to have found a mutation consistent with a further FHL syndrome (FHL6) [35].

1.2.2 Immune deficiency syndromes

Several inborn immune deficiency syndromes are related with the development of HLH. On the one hand, syndromes exist that are associated with hypopigmentation/partial albinism and defects in secretory lysosomes (i.e. cytotoxic granules [36]) [37]: Chédiak-Higashi syndrome (CHS) with a mutation in the gene *LYST* [38], Griscelli-Syndrome type 2 (GS2) with a mutation in the gene *RAB27A* [39], and Hermansky-Pudlak syndrome type 2 (HPS2) with a mutation in the gene *AP3B1* [40]. Given the pathophysiologic overlap in exocytosis related function with the aforementioned FHL syndromes, the distinction within primary HLH according to the presence of immunodeficiency syndromes or FHL is regarded as obsolete [41].

On the other hand, patients suffering from X-linked lymphoproliferative disease (XLP), due to X-chromosomal recessive mutations in either the *SAP* encoding *SH2D1A* gene (XLP-1) or the X-linked inhibitor of apoptosis (*XIAP)* encoding *BIRC4* gene (XLP-2) [42, 43], are prone to develop HLH. Patients with XLP usually show an extreme vulnerability to Epstein-Barr virus (EBV) infections, which therefore often trigger the onset of HLH [44].

An overview of the aforementioned syndromes and genes associated with primary HLH is shown in Table 1 and Figure 1.

Table 1 Overview of primary HLH syndromes (adapted from Usmani, Woda, and Newburger, 2013 [29], and Wegehaupt et al., 2020 [22])

Syndrome	Gene	Protein	Location	Defect
FHL1	Unknown	Unknown	9q21.3-22	Unknown
FHL2	*PRF1*	Perforin	10q21-22	Perforin expression
FHL3	*UNC13D*	Munc13-4	17q25	Granule fusion
FHL4	*STX11*	Syntaxin11	6q24	Granule fusion
FHL5	*STXBP2(UNC18B)*	Munc18-2	19p13.2-3	Granule fusion
FHL6*	*RHOG*	RhoG	11p15.4–p15.5†	Granule fusion
CHS	*LYST*	Lyst	1q42.1-42.2	Vesicle maturation
GS2	*RAB27A*	Rab27a	15q21	Granule docking
HPS2	*AP3B1*			Vesicle trafficking
XLP-1	*SH2D1A*	SAP	Xq24-26	Cytotoxicity against EBV
XLP-2	*BIRC4/XIAP*	XIAP	Xq25	Inhibition of inflammasome activity

* as proposed by Kalinichenko et al. [35]

† as described in Le Gallic and Fort [45]

CHS, Chédiak–Higashi syndrome; *EBV*, Epstein-Barr virus; *FHL*, Familial hemophagocytic lymphohistiocytosis; *GS-2*, Griscelli syndrome type 2; *HPS2*, Hermansky-Pudlak syndrome type 2; *XLP*, X-linked lymphoproliferative disease.

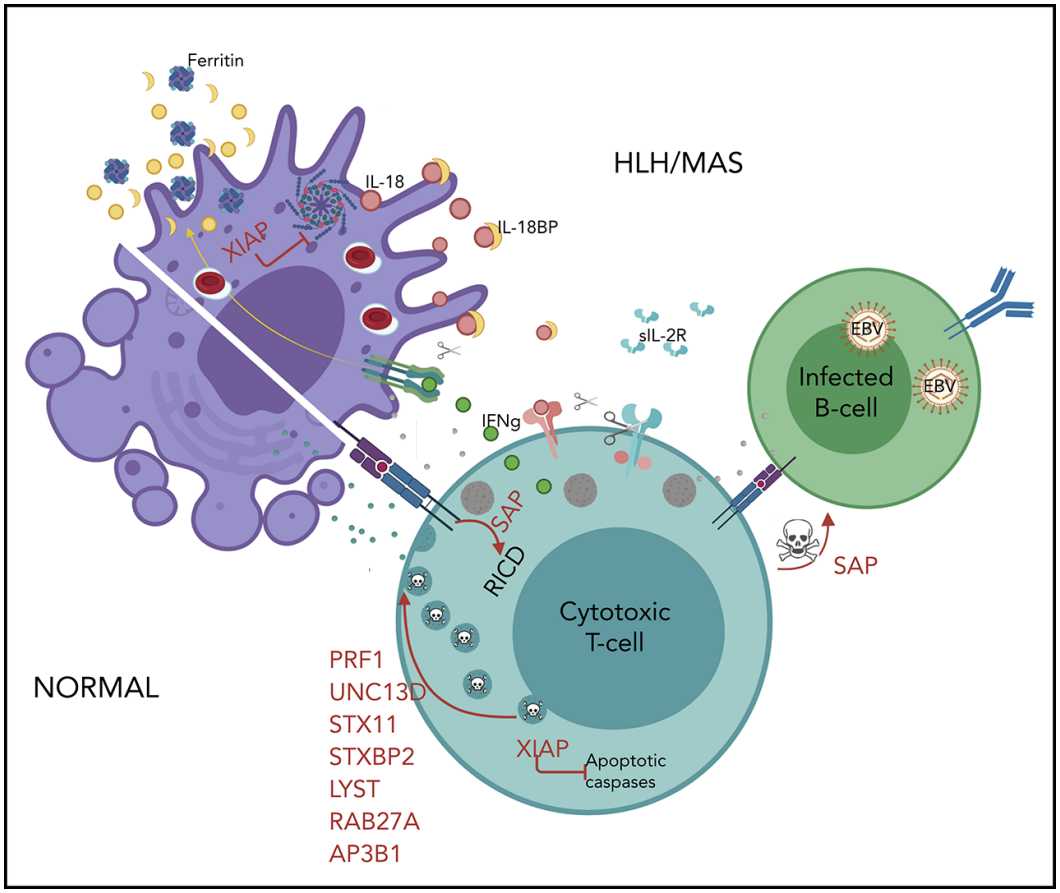

Figure 1 Cellular mechanisms of genetic HLH predisposition. *EBV*, Epstein-Barr virus; *HLH*, Hemophagocytic lymphohistiocytosis; *IFNg,* Interferon gamma; *IL-18BP*,

Interleukin-18-binding protein; *sIL-2R*, alpha chain of the soluble interleukin-2 receptor. (modified from Canna and Marsh, *Pediatric hemophagocytic lymphohistiocytosis* © 2020 by Elsevier [46], license acquired via *Copyright Clearance Center* (license number 5607040326523)).

1.3 Pathophysiology

The identification of aforementioned HLH related genes and proteins marked huge steps forward in the understanding of HLH pathophysiology [3]. Most mutations (with the exception of *XIAP*) affect the granule dependent cytotoxicity of cytotoxic lymphocytes which proved to be essential for immune surveillance and homeostasis [28]. The perforin protein, which is stored in granules of cytotoxic lymphocytes, is playing a central role for HLH pathophysiology. When distributed into the immunological synapse between effector and target cell, the perforin polymers are forming pores in the cell membrane of the target cell [28, 47]. These pores enable the influx of apoptosis inducing granzymes, which are stored in and distributed from the same granules as perforin [28, 47] (Figure 2).

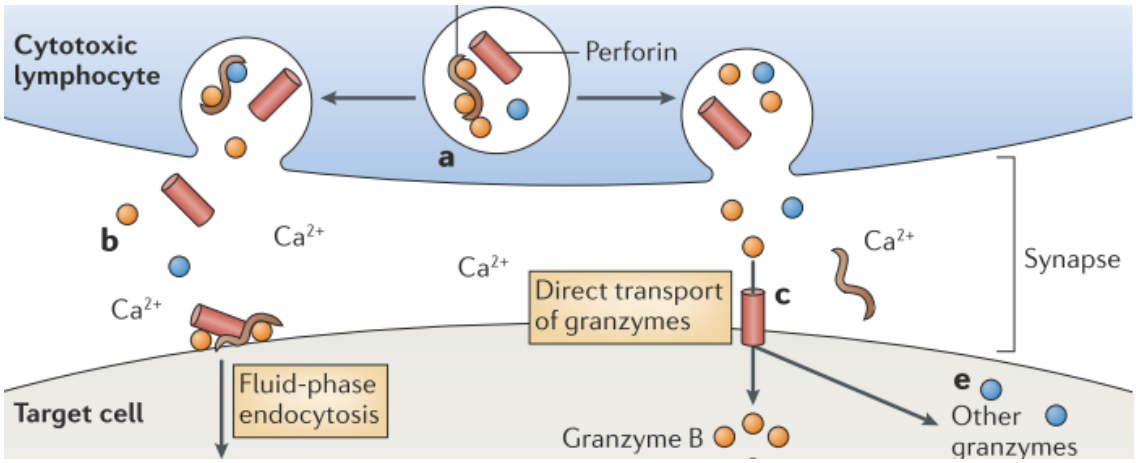

Figure 2 The perforin dependent pathway of cytotoxic granules between effector and target cell. (modified from Voskoboinik, Smyth, and Trapani, *Perforin-mediated target-cell death and immune homeostasis* © 2006 Springer Nature [28], license acquired via *Copyright Clearance Center* (license number 5607041444261)).

In primary HLH, this granule dependent cytotoxicity is impaired because of mutations in genes associated with granule's synthesis and trafficking, or in the gene encoding for perforin itself (*PRF1*) [29].

Therefore, the immune system, despite being activated properly (e.g., by an infectious trigger), fails to adequately eliminate antigen carrying and presenting cells which is followed by an excessive activation and proliferation of T-cells and accompanied by the distribution of different cytokines [22]. The secretion of pro-inflammatory and macrophage activating interferon gamma (IFNγ) has proven to play a pivotal role in the development of HLH-like symptoms in an animal model of perforin-deficient mice [48]. The activated macrophages distribute additional pro-inflammatory cytokines (e.g., interleukin (IL)-1, IL-6, IL-18, tumor necrosis factor alpha (TNFα)), [49] and may start to engulf blood cells. Thus, the eponymous hemophagocytosis is appearing along with the reciprocal activation of cytotoxic lymphocytes [29]. In addition, the negative feedback loop, which is usually being maintained by NK cells and needed for regaining lymphocyte homeostasis, does not work properly either: besides the restriction of NK cell's direct toxicity against activated T-cells, their ability to limit the activation of T-cells is also impaired [50].

The overall result is an overly and enduring distribution of cytokines by the means of a vicious cycle (Figure 3) [46]. Besides the general tissue invasion and organ infiltration by activated T-cells and macrophages, ultimately a cytokine storm causing multi-organ failure and eventually death can arise [51].

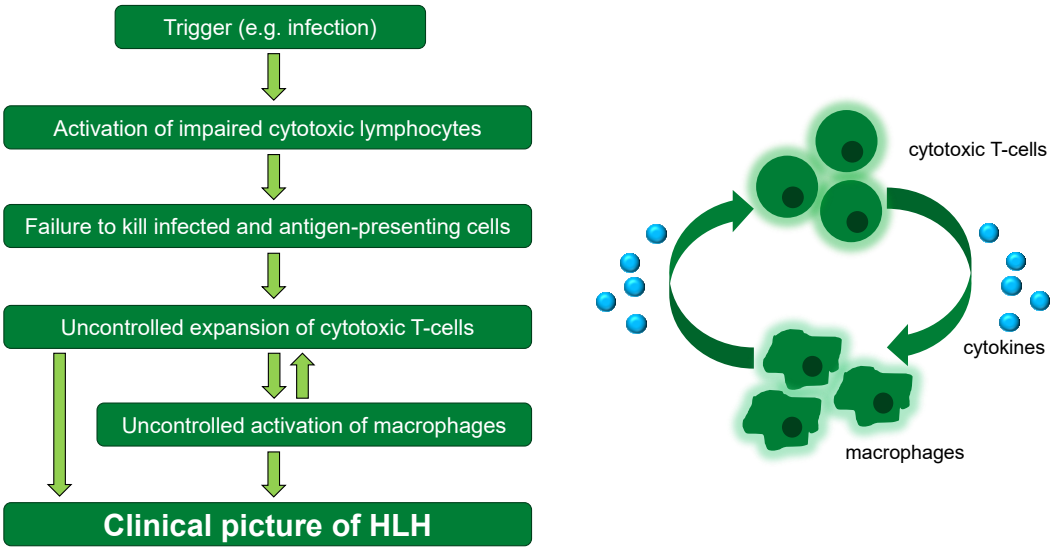

Figure 3 Mechanism of HLH development. (adapted and modified from Janka, 2012 [3])

Recent findings propose that additional pathways, such as mutations concerning macrophage's inflammasome activity, can lead to HLH [52]. Likewise, in patients suffering from XLP-2, granulocyte toxicity is not impaired [42, 43].

1.3.1 Development of distinct symptoms and laboratory findings

The distinct clinical symptoms and laboratory findings of HLH can be explained by the effects of the different cytokines and the tissue infiltration by activated immune cells [6]: fever will arise because of pyrogenic cytokines IL-1, IL-6, and TNFα [3]; hemophagocytosis will result because of macrophages' activation via IFNγ and the invasion of immune cells in the (reticuloendothelial) tissues [53]; cytopenia will develop besides hemophagocytosis [53] via the suppression of hematopoiesis by IFNγ, TNFα, and the heavy subunit of ferritin [3]. Further pathologic laboratory findings can be explained as follows: the secretion of ferritin and plasminogen activator by activated macrophages is leading to hyperferritinemia and hypofibrinogenemia, respectively [3]; hypertriglyceridemia is caused by TNFα associated inhibition of the lipoprotein lipase and an increased synthesis of triglycerides [54]; the elevated levels of the alpha chain of the soluble Interleukin-2 receptor (sCD25) represents the shedding of IL-2 receptor's CD25 subunit (i.e. α-chain) during T-cell activation [55, 56]; hepatosplenomegaly and elevated serum levels of transaminases are the result of organ infiltration by macrophages and activated lymphocytes [57]. In addition, liver cell apoptosis may also be induced by TNFα [3].

1.4 Secondary HLH

Even though secondary HLH is the most common form in older children and adults [3], its exact pathophysiology is still not fully understood because of its overall heterogeneity [41, 58]. It is suspected that an acquired dysregulation of the immune system appears in the context of an infectious, malignant, or autoimmune trigger [51]. Previous immunosuppression such as application of glucocorticoids, chemotherapies, infection with human immunodeficiency virus (HIV), and stem cell/organ transplantation may further disbalance the immune system and therefore increase the risk of developing HLH [59, 60]. However, the reason why only a small number of individuals with aforementioned triggers are developing HLH, remains speculative.

After all, a multifactorial etiology including single nucleotide polymorphisms (SNPs) and hypomorphic mutations in immune system related genes seems likely [7]. In addition, a

temporary dysfunction in immune homeostasis because of low NK cell numbers or previous immune-modulating therapies are suspected to play a role [29]. Of note, monoallelic mutations in FHL related genes were detected in a proportion of 43/240 (18%) secondary HLH cases in a retrospective Italian study [20]. These monoallelic mutations do not allow the diagnosis of primary HLH, but may increase the susceptibility of developing secondary HLH in the presence of an adequate trigger [20]. Accordingly, a threshold model of HLH development including endogenous (e.g., genetic) and exogenous (e.g., infectious) factors, is shown in Figure 4.

Whereas the etiologies and initial pathogenesis in secondary HLH may differ from the primary form, the final cytokine driven pathway and vicious cycle is thought to be identical [61]. Therefore, the initial immunosuppressive treatment approach is similar for both entities [5, 62].

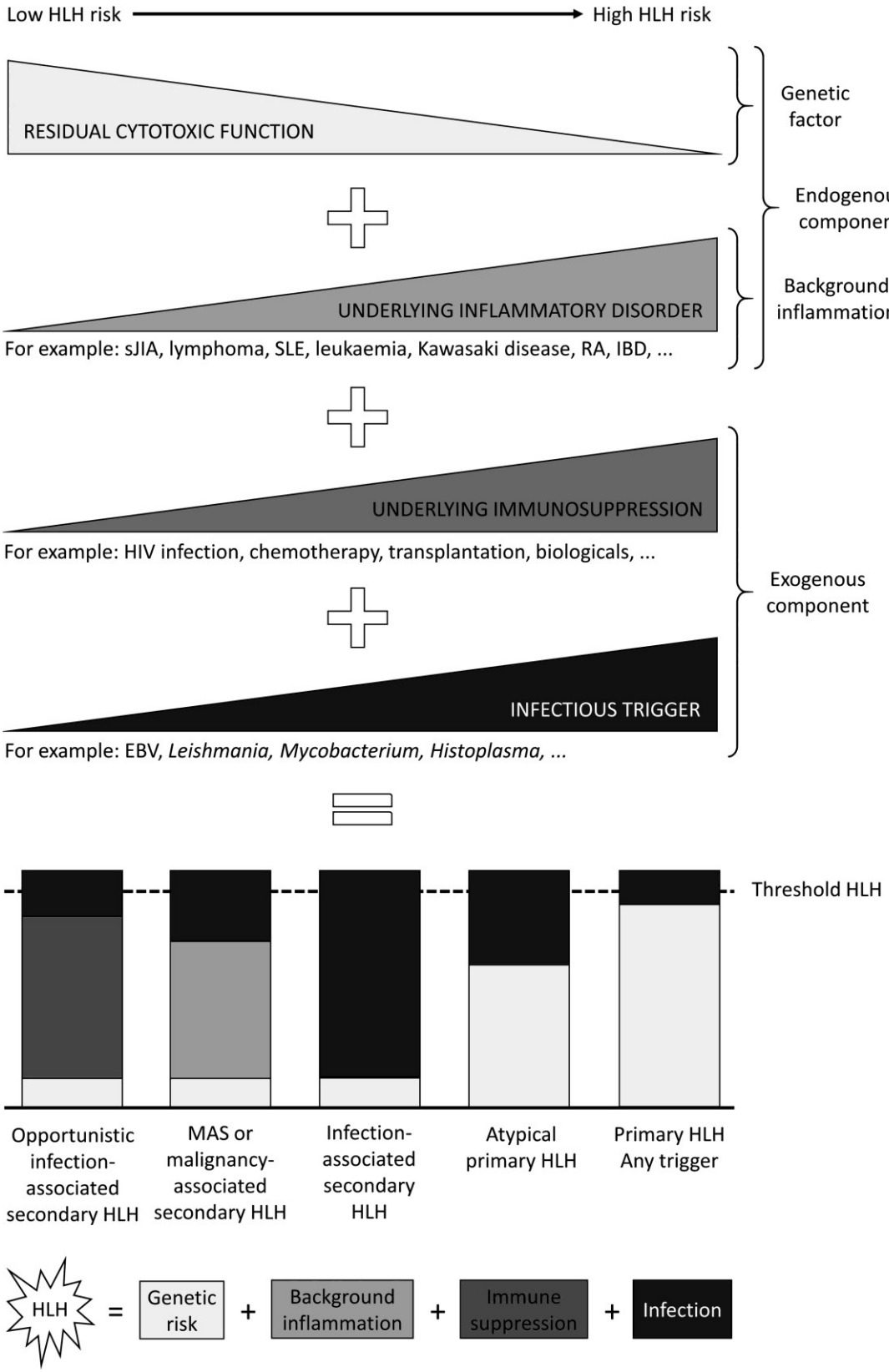

Figure 4 Threshold model of the HLH spectrum. *EBV*, Epstein-Barr virus; *HIV*, human immunodeficiency virus; *HLH,* hemophagocytic lymphohistiocytosis; *IBD*, inflammatory bowel disease; *MAS*, macrophage activation syndrome; *RA*, rheumatoid arthritis;

sJIA, systemic juvenile idiopathic arthritis; *SLE*, systemic lupus erythematosus. (Brisse, Wouters, and Matthys, *Advances in the pathogenesis of primary and secondary haemophagocytic lymphohistiocytosis: differences and similarities* © 2016 John Wiley and Sons [6], license acquired via *Copyright Clearance Center* (license number 5607050279962)).

1.4.1 Infection-associated secondary HLH (I-HLH)

Infections are the most frequent underlying trigger for secondary HLH in children, and are responsible for approximately 50% of HLH manifestation in adults, too [8, 63]. In particular herpes viruses, first and foremost EBV, are capable of triggering HLH [8, 63, 64]. Further DNA viruses, associated with HLH are cytomegalovirus (CMV), herpes simplex virus (HSV), human-herpes viruses 6 and 8 (HHV-6/8), parvovirus B19, and adenoviruses. Also RNA viruses such as HIV, influenza, and recently cases associated with SARS-CoV-2/COVID-19 have been reported in context with HLH [6, 65]. Also infections with bacteria, parasites, and fungi can trigger an HLH episode, but are generally rare: they account for approximately 19, 5, and 3% of I-HLH triggers in adults, respectively [8]. Of note, the proof of an infectious pathogen does not rule out primary HLH, as onset of disease in this entity can be triggered by an infection, too [12, 20, 48, 66].

1.4.2 Malignancy-associated secondary HLH (M-HLH)

Besides infections, malignancies are responsible for most manifestations of secondary HLH in adults [8]. Proportions of M-HLH in adults vary from study to study and can account for up to > 50% of all etiologies [6, 67, 68]. M-HLH may appear before, during, and after diagnosis or therapy of the underlying malignancy [3]. Because of the lower incidence of malignant tumors in children, M-HLH plays a subordinate role in the pediatric setting [58, 63, 69].

The most common neoplasms associated with M-HLH are hematologic ones: in particular lymphoma of the B- and T cell line are capable of triggering HLH. They each account for approximately 1/3 of M-HLH cases [8].

In a Japanese study with over 1200 non-Hodgkin lymphoma (NHL) patients, overall incidence of M-HLH was 2.8% [70]. Patients with T/NK-cell lymphoma had a higher incidence of M-HLH (8.2%) compared to patients with B-cell lymphoma (1.8%), even though the absolute frequency of B-cell lymphoma associated HLH was higher [70]. Further incidences of M-HLH have been described to range from 0.9% (8/887) in patients

with different malignancies [71], up to almost 10% (32/343, 9.3%) in patients with acute myeloid leukemia (AML) who underwent intensive chemotherapy [72]. On the contrary, the occurrence of HLH in patients with solid tumors is rather rare: in an extensive literature research on adult HLH with over 1000 M-HLH cases, an association with solid tumors was only found in about 3%, whereas approximately 94% of cases appeared in context with hematologic neoplasms [8].

1.4.3 Macrophage activation syndrome (MAS-HLH)

Secondary HLH manifesting itself in context with autoimmune and systemic diseases is historically referred to as macrophage activation syndrome (MAS) [73]. The recent *classification of histiocytoses and neoplasms of the macrophage-dendritic cell lineages* subsumes MAS together with primary and secondary HLH in the "H" group and is suggesting the term MAS-HLH for its description [21]. MAS-HLH is appearing frequently in children with systemic juvenile idiopathic arthritis (sJIA) [74]. The two predominant conditions associated with MAS-HLH in adults are systemic lupus erythematosus (SLE) and adult-onset Still's disease (AOSD) – the equivalent to sJIA in adults. Whereas AOSD is associated with a higher MAS-HLH prevalence, ranging from 10 – 15% [75-77], given the overall higher incidence of SLE (MAS-HLH prevalence 0.9 – 9% [78]), more cases of MAS-HLH have been described in context with SLE [78]. Since autoimmune diseases are showing a higher incidence in females, MAS-HLH patients usually are also showing a sex ratio towards women [79, 80].

1.4.4 Idiopathic HLH

When no trigger for the HLH episode can be identified, the term idiopathic HLH is used. The proportion of idiopathic HLH cases in adults is approximately 10% [4]. However, the ratio varies from study to study with percentages ranging from approximately 10% [68, 81], to over 20% [64, 82, 83] or even 30% [84].

1.4.5 Further HLH triggers

Previous immunosuppression is disturbing the balance of the immune system and therefore decreasing the threshold of developing HLH (see Figure 4) [6]. Likewise, manifestations of HLH after previous application of chemotherapies, performance of organ and stem cell transplantation, and in association with HIV infections have been described [59, 72, 85-87]. For this reason, the presence of a previous immunosuppression was also

included in the diagnostic HScore (see chapter 1.5.2) [60]. Recently, HLH manifestations due to the increased use of T-cell activating substances such as chimeric antigen receptor T-cells (CART) have been reported [88-90]. Despite rarely described, HLH-like symptoms may also appear in patients with metabolic disorders [91, 92].

1.5 Diagnostic criteria

An overview of the respective parameters of the two most commonly applied diagnostic algorithms following the HLH-2004 criteria and the HScore is shown in Table 2. Since there is no single specific symptom or laboratory parameter which solely allows the diagnosis of HLH, the concomitance of several clinical, laboratory, and histologic findings is needed to confirm the diagnosis [3].

Table 2 Diagnostic criteria of the HLH-2004 protocol and the HScore (adapted from Henter et al., 2007 [15], and Fardet et al., 2014 [60])

	HLH-2004	HScore
Requirements	≥ 5 criteria or gene defect	> 169 points
Criteria		Point value in brackets ()
Temperature [°C]	Fever	< 38.4 (0) 38.4 – 39.4 (33) > 39.4 (49)
Organomegaly	Splenomegaly	No (0) Splenomegaly or hepatomegaly (23) Hepatosplenomegaly (38)
Cytopenia*	≥ 2 of 3 lineages	1 lineage (0) 2 lineages (24) 3 lineages (34)
Hemophagocytosis	In bone marrow / spleen / lymph nodes	Absent (0) In bone marrow aspirate (35)
Ferritin [µg/L]	≥ 500	< 2000 (0) 2000 - 6000 (35) > 6000 (50)
Fibrinogen [g/L] †	≤ 1.5	≤ 2.5 (30) > 2.5 (0)
Triglycerides [mg/dL] †	≥ 265	< 132.7 (0) 132.7 – 354 (44) > 354 (64)
NK cell activity	Low / absent	/
sCD25 [U/mL]	≥ 2400	/

AST [U/L]	/	< 30 (0) ≥ 30 (19)
Immunosuppression #	/	No (0) Yes (18)

AST, Aspartate transaminase: *NK*, Natural killer; *sCD25*, alpha chain of the soluble interleukin-2 receptor.

* Cutoffs: HLH-2004: hemoglobin < 9.0 g/dL, platelets < 100 /nL, neutrophils < 1.0 /nL; HScore: hemoglobin ≤ 9.2 g/dL, platelets ≤ 110 /nL, white blood cell count ≤ 5.0 /nL

† Presence of hypofibrinogenemia and/or hypertriglyceridemia condensed into one HLH-2004 criterium

HIV positive or receiving long-term immunosuppressive therapy (i.e., glucocorticoids, cyclosporine, azathioprine)

1.5.1 HLH-2004

The most important and widespread diagnostic criteria are following the scheme of Histiocytic Society's prospective HLH-2004 trial [23]. For a positive HLH diagnosis the proof of either a disease causing mutation in HLH related genes or the simultaneous presence of at least five out of the following eight criteria must be present: (1) fever, (2) splenomegaly, (3) cytopenia of at least two lineages, (4) hypertriglyceridemia or hypofibrinogenemia, (5) hyperferritinemia, (6) hemophagocytosis in bone marrow, spleen or lymph nodes, (7) low/absent NK cell activity, and (8) elevated levels of sCD25 [15].

It is important to note that the HLH-2004 criteria were developed for a population of pediatric patients without underlying malignancies or rheumatic diseases [93]. Therefore, the adoption of these criteria for all patients with suspected HLH, particularly in the adult setting – where etiologies and laboratory aberrations sometimes differ substantially from the pediatric one – may pose problems. One often cited issue refers to the comparably low cutoff point of ferritin at 500 µg/L (see chapter 4.2.3). In addition, it appears rather arbitrary why hypertriglyceridemia and hypofibrinogenemia are condensed into one criterium [94]. Lastly, the measurement of NK cell activity requires specialized laboratory equipment and is therefore not readily available in most institutions [23]. Since pathologic results in functional tests such as NK cell activity are usually rare in adults, they are not generally recommended during the initial diagnostic work-up [61, 62].

Despite the aforementioned limitations, the HLH-2004 criteria remain the most important and widely used ones in diagnosing adult HLH patients [61, 62].

1.5.2 HScore

In order to address some of the shortcomings of the HLH-2004 criteria, a French working group introduced the HScore in 2012 [60]. In contrast to the HLH-2004 criteria, the HScore was developed and evaluated in adult patients with secondary HLH. The HScore includes nine readily available laboratory and clinical parameters: (1) previous immunosuppression (e.g., HIV-infection, long term immunosuppressive therapy), (2) elevated body temperature, (3) organomegaly, (4) number of cytopenia, (5) hyperferritinemia, (6) hypertriglyceridemia, (7) elevation of aspartate transaminase (AST), (8) hypofibrinogenemia, and (9) features of hemophagocytosis in bone marrow aspirates [60]. Whereas most of these parameters are also included in the HLH-2004 criteria, differences arise in the height of the cutoff values and the fact that each criterion has a specific correlated weight, represented in a corresponding point value. The cutoffs and point values were calculated by using a logistic regression model [60]. In addition, the inclusion of AST, even though given a low cutoff, represents the typical hepatic involvement in the course of multi-organ infiltrating HLH [5].

After summing up all point values, the HScore ranges from 0 to 337 points, which reflect a probability of positive HLH diagnosis between < 1% (≤ 90 points) and > 99 % (≥ 250 points). The recommended cutoff, of which 90% of patients have been classified correctly, is 169 points (sensitivity 93%, specificity 86%) [60]. In a study with MAS-HLH patients, an adapted cutoff at 190.5 points has been recommended [95].

The HScore can also be addressed online for the use in the clinical setting (see Supplement).

1.5.3 Diagnostic approach

An important preliminary step to diagnosing HLH is being aware of the syndrome itself: in patients with a triad of prolonged fever, cytopenia, and splenomegaly, suspicion of a possible HLH episode should arise [36]. During the succeeding diagnostic work-up, three questions have to be answered subsequently: (1) Are the laboratory and clinical parameters compatible with HLH? – (2) Can an underlying trigger be identified? – (3) Does a genetic background exist? [41].

1.5.3.1 Anamnesis and clinical examination

Anamnesis should contain questions about recent infections, known malignancies, underlying autoimmune/rheumatic diseases, previous immunosuppressive therapy, vaccinations, family history of malignancies and HLH, and travel history (with regard to an incubation period of up to and even longer than one year in leishmaniasis, also travel that took place longer ago may be relevant [96]) [97].

A basic internal/cardiopulmonary examination under consideration of hepatosplenomegaly, lymphadenopathy, bleedings, accompanied by an inspection of the skin and neurologic functions should be performed and repeated regularly in all patients [97].

1.5.3.2 Laboratory work-up

Laboratory work-up should include all parameters of the HLH-2004 criteria and the HScore: complete blood count (hemoglobin, platelets, leukocytes, neutrophil granulocytes), ferritin, sCD25, triglycerides, fibrinogen, and AST. Further useful parameters include lactate dehydrogenase (LDH), c-reactive protein (CRP), procalcitonin (PCT), albumin, bilirubin, gamma-glutamyltransferase (GGT), alkaline phosphatase (AP), creatinine, coagulation study (including international normalized ratio (INR), prothrombin time, (activated) partial thromboplastin time (a)PTT, d-dimer), lactate, urinalysis [97], immunoelectrophoresis, and a pregnancy test in women of childbearing age [98]. Immunologic assays, such as NK cell activity can be considered in patients where hereditary disease is suspected (e.g., in cases of positive family history of HLH, consanguinity, young male patients with EBV infection, recurrent HLH episodes), but are not mandatory in the broad adult population, in particular in the initial work-up [61]. Of note, lymphocyte degranulation assays of perforin and CD107a are preferred in diagnosis compared to NK cell degranulation due to their higher sensitivity in screening for genetic HLH causes [99, 100].

Depending on the laboratory capacities, the return time of sCD25 may take several days to weeks, especially when the samples have to be sent to external laboratories [101]. With regard to a shorter turnaround time it is recommended to measure sCD25 by using a chemiluminescent immunoassay (CLIA), rather than an enzyme-linked immunosorbent assay (ELISA) [56]. The CLIA also allows a broader measuring range, which is extremely important in adult HLH patients, since sCD25 values can be elevated very strongly (see Figure 16).

1.5.3.3 Search for underlying trigger

Search for an underlying infectious trigger should be performed and repeated in all patients, even when a possible trigger has already been identified [69].

The infectious work-up should include serology studies and viral loads for EBV, CMV, HSV, HHV-6, HHV-8, HIV, parvovirus, adenovirus, varicella zoster virus, influenza, SARS-CoV-2, a hepatitis screening and an *Aspergillus* antigen ELISA. Furthermore, cultures of blood, urine, sputum, and – if applicable – bronchoscopy (possibly including tuberculosis) should be carried out. If suspicion arises, for example because of previous stays in an endemic region [8], leishmaniasis serology and polymerase chain reaction (PCR) in bone marrow [96], as well as a thick blood smear for detecting malaria, should be performed [97].

Malignancy work-up should include peripheral flow cytometry, peripheral blood and bone marrow smear review, punction of suspected tissues (lymph node, liver, spleen, cutaneous lesions), and possibly metabolic imaging such as positron-emission tomography (PET) computed tomography (CT) [97, 101].

In case of suspicion of an autoimmune/rheumatologic disease the measurement of antinuclear antibodies (ANAs), anti-neutrophil cytoplasmic antibodies (ANCAs) and anti-double stranded DNA (anti-dsDNA) antibodies should be considered [101].

1.5.3.4 Imaging and interventions

Bone marrow examination with histopathology, flow cytometry, and molecular genetics should be performed in order to detect hemophagocytic figures or an underlying malignancy [101].

Further imaging should include chest-x-ray (pneumonia?), abdomen sonography (hepatosplenomegaly, lymphadenopathy, effusions/hydrops?), CT (lymphadenopathy, infection, tumor?). PET-CT can be considered in case of a yet unknown trigger or suspicion of lymphoma [102, 103]. In patients with neurologic symptoms, cerebrospinal fluid (CSF) puncture (cave: bleeding risk!) and cranial magnetic resonance imaging (MRI) should be performed [69].

After HLH diagnosis is confirmed, genetic testing can help to assess recurrence risk or HLH risk in family members. Either FHL related multi-gene panels or – if available and desired – WES can be processed [100].

A flow diagram of a possible diagnostic and therapeutic work-up is shown in Figure 5.

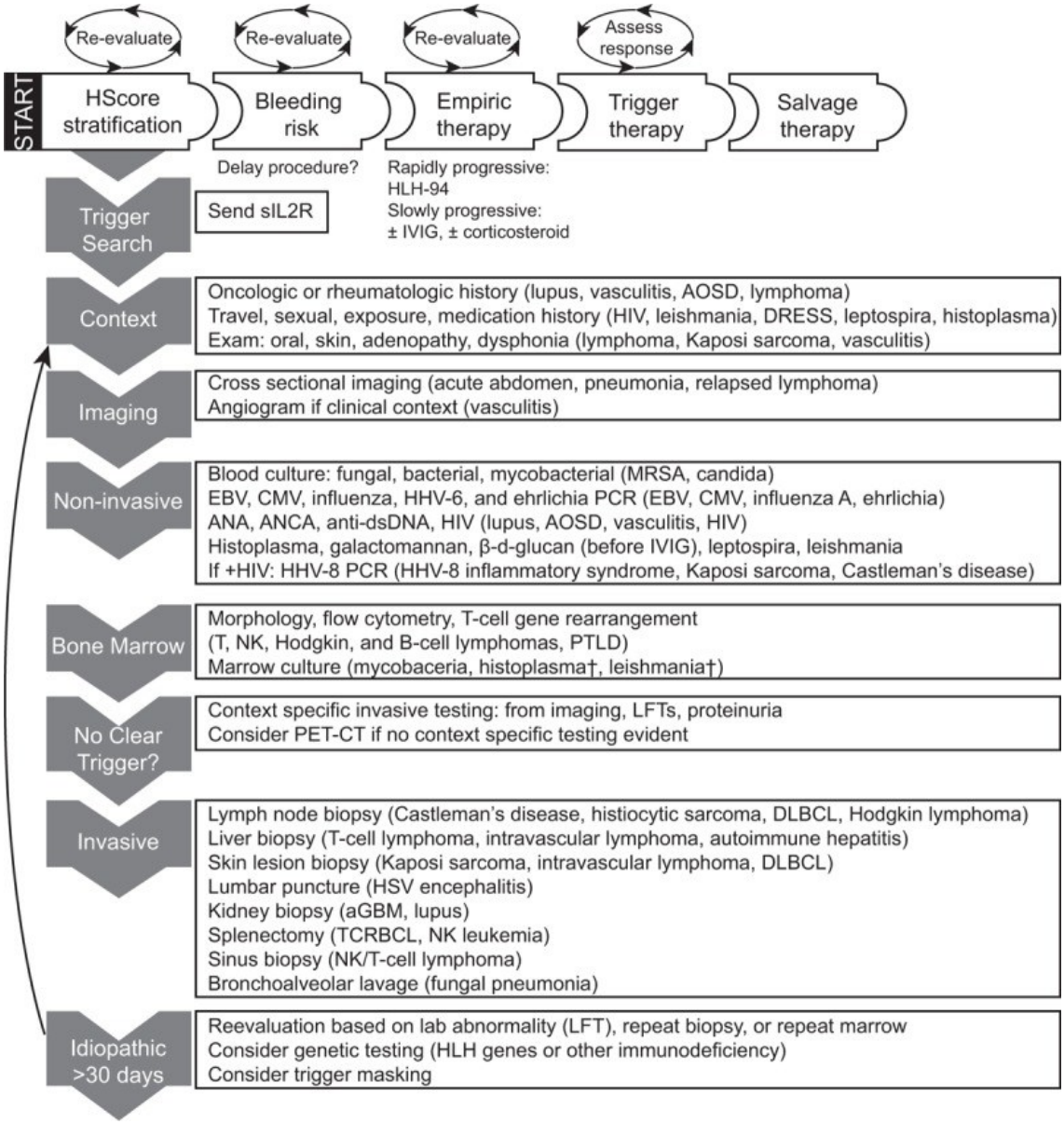

Figure 5 Diagnostic and therapeutic work-up in HLH patients. *aGBM*, anti-glomerular basement membrane disease; *AOSD*, Adult-onset Still's disease; *CMV*, cytomegalovirus; *DLBCL*, diffuse large B-cell lymphoma; *DRESS*, drug reaction with eosinophilia and systemic symptoms; *EBV*, Epstein–Barr virus; *HHV*, human herpes virus; *HIV*, human immunodeficiency virus; *HSV*. herpes simplex virus; *IVIG*, intravenous immunoglobulin; *LFT*, liver function tests; *MRSA*, methicillin resistant *staphylococcus aureus*; *NK*, natural killer cell; *PCR*, polymerase chain reaction; *PTLD*. post-transplant lymphoproliferative disease; *sIL2R*, alpha chain of the soluble interleukin-2 receptor; *TCRBCL*, T-cell rich large B-cell lymphoma. (Merrill et al., *A prospective quality improvement initiative in adult hemophagocytic lymphohistiocytosis to improve testing and a framework to facilitate trigger identification and mitigate hemorrhage from retrospective analysis* © 2018

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1.5.4 Differential diagnosis sepsis

HLH and sepsis share a common phenotype and therefore are often hard to differentiate in the clinical setting [104, 105]. Basically, all HLH-2004 criteria can also be met by patients with active sepsis, including reduced NK cell activity and hemophagocytosis [104, 105]. However, because of diverging therapeutic approaches (immunosuppressive HLH therapy vs. anti-infectious sepsis therapy) it is extremely important to distinguish between these two hyperinflammatory syndromes [105].

Suspicion of HLH should arise in sepsis patients with deteriorating clinical condition under broad-spectrum antibiotic and supportive therapy [106]. Since the occurrence of bi-/pancytopenia, hypertriglyceridemia, and hypofibrinogenemia is less common in sepsis patients, these parameters may be helpful for the differentiation between sepsis and HLH [105]. Furthermore, sCD25 serum levels are generally less elevated in context with sepsis compared to HLH [105, 107].

1.6 Therapy

Given the heterogenous etiologies of secondary HLH, the establishment of an uniform treatment protocol, as it is recommended for patients with primary HLH, has not been achieved yet [58], nor does it seem possible [61]. Therefore, the application of different treatment strategies for separate underlying conditions such as malignancies, infections, and autoimmune diseases should be considered [23]. A treatment algorithm for adults is shown in Figure 6.

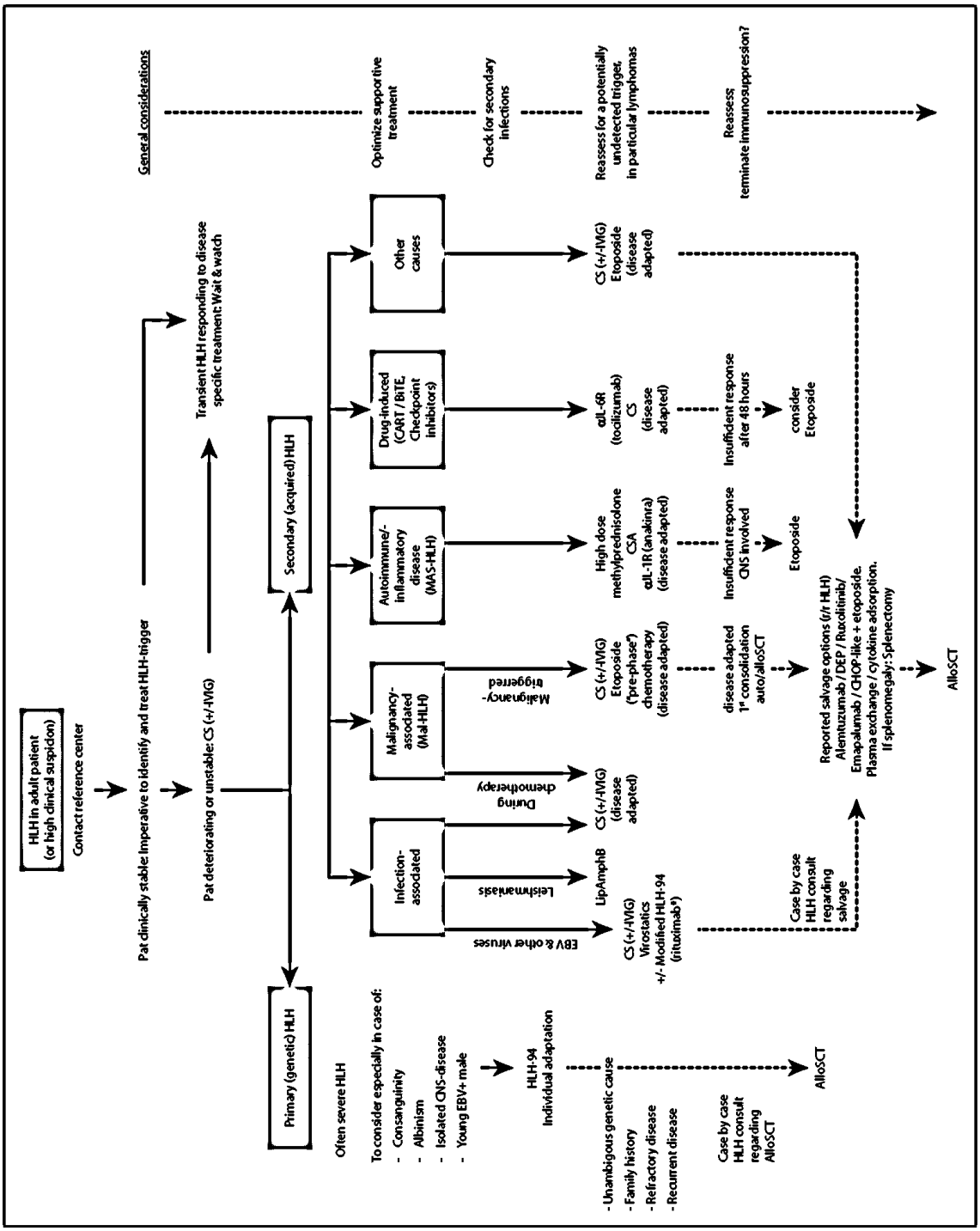

Figure 6 Treatment algorithm for adult HLH patients. *BiTE*, bispecific T-cell engager; *CART*, chimeric antigen receptor T-cells; *CHOP*, cyclophosphamide, doxorubicin, vincristine, prednisolone; *CS*, corticosteroids; *DEP*, doxorubicin, etoposide, methylprednisolone; *IVIG*, intravenous immunoglobulin; *Pat*, patient. (modified for better readability from La Rosee et al., *Recommendations for the management of hemophagocytic lymphohistiocytosis in adults* © 2019 by Elsevier [61], license acquired via *Copyright Clearance Center* (license number 5607050846868)).

To sum it up, the general treatment approach in secondary HLH can be viewed as follows: the early suppression of the possibly lethal hyperinflammatory state by administering immunosuppressive, T-cell- and macrophage depleting therapies is of primary importance [3]. If an underlying trigger, such as an infection can be detected, additional targeted therapy should be applied [97]. Because of chemotherapy's toxicity, the treatment of an underlying malignancy may not be immediately possible in states of florid HLH disease activity and has therefore to be delayed until clinical stabilization has been reached in some cases [98, 108]. On the other hand, the therapy of the trigger itself is sometimes sufficient to treat the HLH episode (e.g., in visceral leishmaniasis) [3].

There is a therapeutic dilemma occurring in severely ill patients, for whom a massive immunosuppression may be as lethal as the underlying hyperinflammatory state itself [3]. Furthermore, by depleting the remaining immunologic functions through HLH therapy, patients are prone to secondary infections. Thus, the administration of prophylactic antimicrobial agents against *pneumocystis jirovecii*, fungi, and viruses is recommended [61, 97]. In addition, a good supportive care approach with sufficient substitution of blood products and organ supporting measurements should be applied, if necessary [97].

1.6.1 HLH-94 protocol

The recommended first line therapy both for primary and secondary HLH is based on the treatment protocol of the pediatric HLH-94 trial [4, 61], which managed to improve long-term survival in children from < 5% [13] to > 50% [14]. Whereas the aim in primary HLH is to stabilize and bridge patients until curative allogeneic stem cell transplantation (allo-SCT) can be performed [12], the HLH-94 regimen is also serving as the basic treatment scheme in secondary and adult HLH patients [61]. The combined immunochemotherapeutic regimen consists of a basis of dexamethasone and etoposide, with the facultative addition of cyclosporine and an intrathecal methotrexate therapy [12]. Dexamethasone was chosen because of its ability to penetrate the blood brain barrier more easily and its longer half-life in the central nervous system (CNS) in comparison to other glucocorticoids such as prednisolone [109]. Of note, all three main agents of the HLH-94 protocol affect T-cells: etoposide, a topoisomerase II inhibitor [110], showed a selected depletion of activated T-cells in the mouse model [111]; glucocorticoids, besides reducing the cytokine distribution, also act cytotoxic on activated T-cells [22], and cyclosporine is reducing the activation and proliferation of T-cells [22].

In the updated HLH-2004 protocol, cyclosporine A was applied right away during induction therapy and prednisolone was added to the intrathecal therapy (Figure 7) [15]. However, since no significant improvement in survival was achieved [93], the HLH-94 protocol remains the recommended first line therapy [4]. Even more so, since severe side effects, such as posterior reversible encephalopathy syndrome (PRES), have been reported in context with cyclosporine therapy [112, 113].

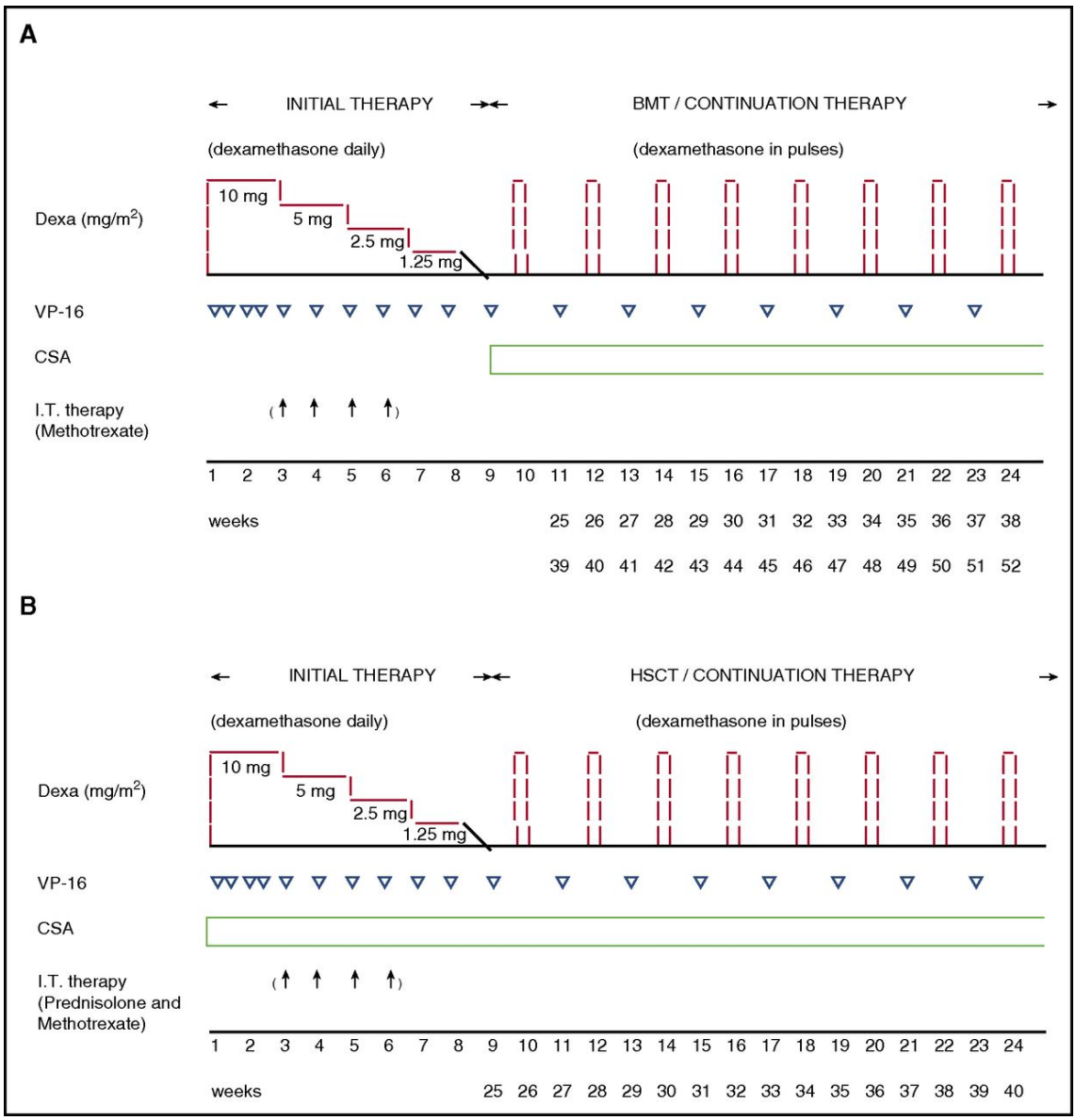

Figure 7 HLH-94 (A) and HLH-2004 (B) treatment protocols. *BMT*, Bone marrow transplantation; *CSA,* Cyclosporine A; *Dexa*, Dexamethasone; *HSCT*, hematopoietic stem cell transplantation; *I.T.*, intrathecal; *VP-16*, Etoposide. (Bergsten et al., *Confirmed efficacy of etoposide and dexamethasone in HLH treatment: long-term results of the cooperative*

HLH-2004 study © 2017 by Elsevier [93], license acquired via *Copyright Clearance Center* (license number 5607051093191)).

1.6.1.1 Initial therapy

Therapy starts with the administration of 10 mg/m² dexamethasone daily and doses of 150 mg/m² etoposide twice a week [12] (Figure 7). In less severe cases, the sole application of dexamethasone, possibly with the addition of intravenous immune globulins (IVIGs), can be sufficient to treat the HLH episode [57]. In patients with progressive neurologic symptoms or persisting pathologic findings in CSF, despite two weeks of systemic treatment, an intrathecal methotrexate therapy once a week should be considered [12, 61]. Of note, because of interindividual differences in clinical condition, the non-dosage adjusted application of dexamethasone and etoposide is not always recommended nor possible. Particularly in adults with deranged clinical condition, dosages often need to be lowered to avoid excessive toxicity [61, 93]. Therefore, the application of etoposide once a week with a reduced dosage from 50 – 100 mg/m² can be considered [4, 61]. An adapted treatment recommendation with the inclusion of IVIGs for adult HLH patients is shown in Figure 8.

Depending on the clinical response, initial therapy may be terminated before or extended beyond eight weeks [4].

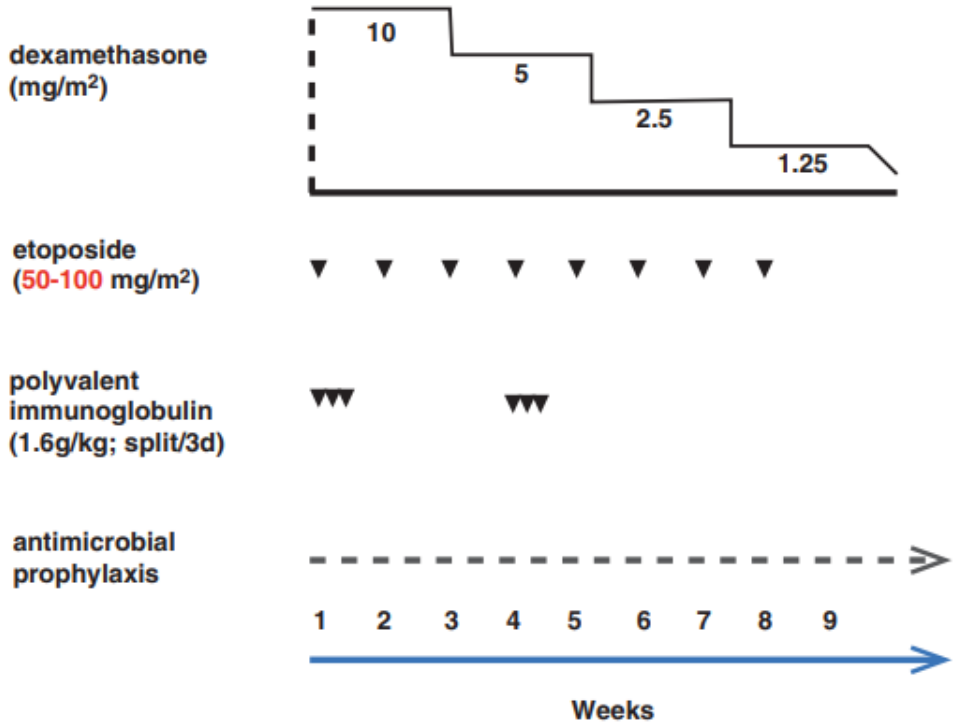

Figure 8 Suggested treatment protocol in adult HLH patients. (La Rosée and Machowicz, *HLH in Adults* © 2018 Springer Nature [62], license acquired via *Copyright Clearance Center* (license number 5607060072930)).

1.6.1.2 Continuation therapy

If patients with secondary HLH show clinical and laboratory remission within the first eight weeks of treatment and no genetic cause for HLH is suspected, the treatment can be terminated [12, 41, 93]. In case of a genetic HLH syndrome, maintenance therapy consisting of cyclosporine and pulses of dexamethasone plus etoposide should be applied, until a curative alloSCT can be performed (Figure 7) [12].

1.6.2 Salvage Therapy

If therapy response within the first 2 – 3 weeks after induction therapy is insufficient (refractory HLH), the application of a salvage therapy regimen should be considered [5]. Besides the patient's clinical presentation, an increase in serum ferritin and sCD25 levels during initial therapy may reflect lacking therapy response [114-116]. In the HLH-94

trial, approximately 30% of the population were showing insufficient therapy response [114].

1.6.2.1 Doxorubicin, etoposide, methylprednisolone (DEP)-regimen

The DEP-regimen consists of doxorubicin, etoposide, and methylprednisolone and was introduced and evaluated in one of the few prospective treatment trials of adult HLH [114]. The study included 63 adult patients (≥ 18 years) with refractory HLH of different etiologies, mainly EBV-HLH and M-HLH [114]. Prior to receiving the DEP-regimen, all patients were treated for at least two weeks with glucocorticoids and etoposide according to the HLH-94 protocol, without achieving partial response [114]. Eventually, a response to the DEP-regimen was achieved in more than 75% (48/63) of patients, with more than 25% (17/63) reaching a state of complete response [114]. However, overall mortality still succeeded 50% and 19/48 (40%) patients, who achieved at least a partial response, died [114].

1.6.2.2 Further agents

The application of comparably new and often biological based agents such as the IL-1 receptor antagonist anakinra [117], the Janus kinase (JAK) inhibitor ruxolitinib [118-120], and monoclonal antibodies against the IL-6 receptor (tocilizumab) [121], IFNγ (emapalumab) [122], CD52 (alemtuzumab) [123], sCD25 (daclizumab) [124], and TNFα (infliximab) [125] may be considered in selected cases. The data on most of these substances is scarce, especially in the adult population, but they may serve as a valuable third line therapy attempt in refractory cases.

In addition, the application of a cytokine adsorber in HLH patients with an established external circuit may be a viable option. Some case description showed a therapy response combined with a decline in cytokine levels and bilirubin in HLH patients [126-128]. Since in severe cases with multiple-organ failure, hemodialysis usually has to be installed anyways, the additional application of a cytokine adsorber should be considered in these patients.

1.6.3 Stem cell transplantation (SCT)

alloSCT remains the only curative treatment option for patients with primary HLH [4]. alloSCT can furthermore be considered in children with CNS involvement and in episodes of recurrent/refractory HLH or enduring impairment of NK cell function [5]. However, with respect to adults, where homozygous mutations in HLH related genes are scarce and clinical conditions often deranged, alloSCT is playing a subordinate role and remains an option in selected cases (e.g. for patients with severe genetic aberrations, late onset FHL syndromes, or relapsed/refractory HLH, especially in context with chronic active EBV infection and refractory lymphoma [4]) [23]. In states of florid HLH disease, SCT should not be performed with regard to the inflammatory state, which leads to an increased risk of developing graft-versus-host disease [97].

The application of a reduced-intensity conditioning showed significant advantages concerning overall survival in pediatric patients [129], whereas no significant improvements could be found in adults [130, 131].

Sometimes, preemptive alloSCT in asymptomatic patients with severe underlying genetic mutations (e.g., *PRF1* with complete loss of perforin expression), particularly in presence of symptomatic siblings, can be considered [4, 132]. Therefore, the genetic testing of unaffected siblings in case of primary HLH is recommended [133]. In addition, it is important to rule out the existence of the same/further mutations in FHL related genes, when looking for a suitable stem cell donor within the index patient's family [61, 133].

1.7 Prognosis

In cases of untreated primary HLH, median survival ranges between one and two months: only 12% of patients survived longer than six and 5% longer than twelve months, respectively [13]. Through the introduction of the etoposide containing HLH-94 and HLH-2004 protocols, estimated five-year survival in the pediatric setting reached 54% and 61%, respectively [93]. In adults, overall mortality ranges between 20 and > 80%, depending on the composition of the study population [62].

1.7.1 Prognosis according to etiology

M-HLH is associated with the worst prognosis of all secondary HLH triggers: a median overall survival of < 1.5 months has been described [67, 134]. In contrary, patients with MAS-HLH generally show the best long-time survival of all secondary HLH cases [8, 23, 64, 81]. The prognosis of patients with I-HLH is usually between HLH triggered by malignancies and autoimmune/systemic diseases [81]. Prognosis of EBV-HLH is generally worse than HLH caused by other infectious pathogens [64]. Since the identification of an underlying trigger is important in order to enable its targeted therapy, patients with idiopathic HLH generally show comparably poor outcomes [8]. Of note, a possible undetected malignant trigger, which can be hidden by active HLH [135, 136] or HLH therapy [100] may contribute to the poor prognosis of idiopathic cases. Ramos et al. described in their extensive literature research on adult HLH a mortality rate of 41% in 1109 patients of different etiologies [8].

1.7.2 Prognostic parameters

Selected adverse prognostic factors in previous studies have been older age [64, 81, 83, 87, 115, 137-143], male sex [83, 144], malignancy-associated HLH [67, 82, 134, 138, 141, 143, 145], elevated levels of alanine transaminase (ALT) [146], ferritin [64, 134, 137, 140, 146], sCD25 [147-150], LDH [141, 144], triglycerides [141], bilirubin [87]; lowered levels of platelets [64, 81, 83, 84, 87, 137, 138, 141, 146, 151], albumin [67, 81, 115], fibrinogen [142, 152-154], hemoglobin [137, 142] neutrophils [81], natrium [115]; an increase of ferritin during disease course [115, 155, 156], EBV association [64, 151], no etoposide therapy [138], presence of hemophagocytosis in bone marrow [142], hepatomegaly [115], and pleural effusion [151].

1.8 Research question

The aim of this study was to increase the understanding of the rare hyperinflammatory syndrome HLH by retrospectively collecting data concerning epidemiology, etiology, symptoms, diagnostic procedures including metabolic imaging, applied therapies, clinical outcome, and prognosis of adult patients with HLH treated in Munich. Besides increasing the amount of available epidemiologic data, an improvement of the clinical management and therefore outcome of affected patients was the aim of the project.

Parts of the following chapters have been published in *sCD25 as an independent adverse prognostic factor in adult patients with HLH: results of a multicenter retrospective study* [2].

2. Material and methods

2.1 Ethics statement

Approval of the study was granted by the ethics committee of the medical faculty of LMU Munich (project number 20-0932). Because of the retrospective approach of the study and the anonymous data collection written informed consent was waived. [2]

2.2 Study population

All data were obtained from patients who were treated in the participating hospitals (University Hospital LMU Munich, Red Cross Hospital Munich, Munich Clinic Schwabing, Klinikum rechts der Isar, Technical University of Munich (TUM)) within the time period of 01/01/2010 to 01/31/2021 which also represents the study period. The identification of suitable patients was conducted by the medical statistics departments of the respective clinics by using HLH-associated ICD-10 codes (D76.-). An overview of the search algorithms and the included patients per hospital is shown in Figure 9. [2]

All adult patients (≥ 18 years) who met at least five HLH-2004 criteria or simultaneously presented with an HScore > 200 plus four positive HLH-2004 criteria were included for further analysis. Sixty-one individuals met at least one of these requirements. According to the HLH-2004 criteria, one further patient was included in the analysis because of a mutation in the *XIAP*-gene, even though he did not fulfill aforementioned criteria (four positive HLH-2004 criteria plus an HScore of 107). The amount of HLH-2004 criteria and accompanying height of the HScore per patient is shown in Figure 10. [2]

For the assessment of the diagnostic criteria, the corresponding minimum/maximum laboratory value, which was obtained until 14 days before the initiation of a HLH specific therapy, was used. In case of missing data for this period, the first available value after the beginning of treatment was notated. Only the data of the first HLH-associated hospital

stay per patient were included. Exceptions are genetic and immunologic findings, which were included independently from the time of their assessment. [2]

Part of the data of five patients (three treated at University Hospital LMU, and one each at Red Cross Hospital Munich, and at Munich Clinic Schwabing) had been reported to the German HLH-register until 07/2017 and have therefore also been previously published in "*Hemophagocytic lymphohistiocytosis in adults: collaborative analysis of 137 cases of a nationwide German registry*" [81]. [2]

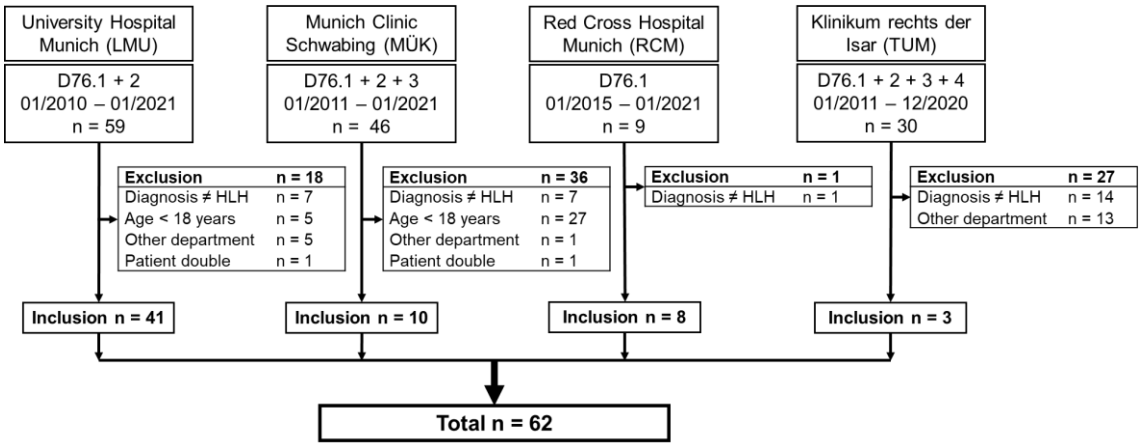

Figure 9 CONSORT diagram showing search related ICD-10 codes and timespans, numbers and reasons for exclusions, and the total number of included patients per hospital (Wimmer et al., *sCD25 as an independent adverse prognostic factor in adult patients with HLH: results of a multicenter retrospective study* © 2023 by Elsevier [2], permission of the publisher as author of the article not required).

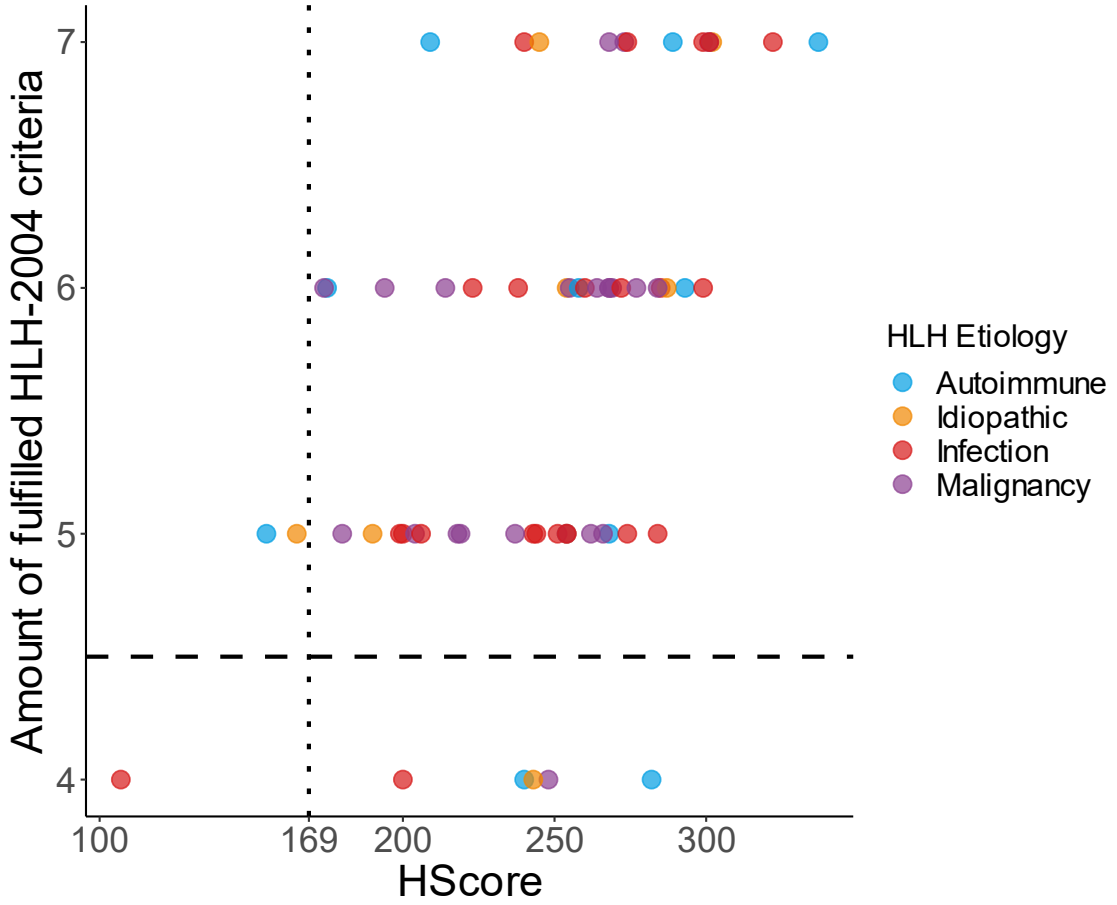

Figure 10 HLH-2004 criteria and HScore per patient [n = 62]. According to the HLH-2004 study protocol, at least five out of eight criteria must be present for an HLH diagnosis [15]; the recommended cutoff of the HScore is 169 points [60]. The patient who did not meet any of the aforementioned criteria (four HLH-2004 criteria, 107 points in the HScore) had a proven mutation in the *XIAP* gene.

2.3 Data collection

The electronic, and in some cases additional printed, patient files of all suspected HLH cases were reviewed manually and the data of interest entered in several excel files. Only data obtained before the end of the study period (01/31/2021) were collected. [2]

Collected data fields include epidemiologic and standard clinical data (sex, age, weight, height, date of hospital admission and dismissal, date of onset of symptoms, date of HLH diagnosis, date of HLH-directed therapy, outcome (survival/death), date and cause of death, date of the last follow-up, symptoms, initial symptoms and complications, need of

ventilation and hemodialysis, duration of intensive care unit (ICU) treatment, etiologic HLH subgroup/most likely HLH trigger, type of and duration of previous immunosuppressive/immune-activating therapies, underlying autoimmune disease, malignancy or infection, state of previous/recent EBV-infection, occurrence of fever, splenomegaly, hepatomegaly, and lymphadenopathy); results of microbiologic, virologic and genetic analyses; date and results of metabolic imaging; date and results of bone marrow and CSF examinations; applied therapies and medication. The previously mentioned data were coded by numbers and entered in a comprehensive excel file for all patients.

In addition, two individual excel files per patient were created: one included all relevant laboratory parameters (albumin, ALT, antithrombin, AP, AST, (direct) bilirubin, CRP, creatinine, ferritin, fibrinogen, GGT, hemoglobin, immunoglobulins A (IgA), G (IgG), and M (IgM), IL-6, INR, lactate, LDH, leukocytes, natrium, (absolute) neutrophil count, (a)PTT, platelet count, PCT, sCD25, and triglycerides) per date; the second file resembles a patient curve/fever chart and includes pre-existing illnesses and treatments, information on the treating hospital department, body temperature, symptoms, complications, applied therapies, interventions, results of imaging procedures, virologic/microbiologic/pathologic/genetic/functional measurements, and transfused blood products per date.

The combination of a comprehensive excel file for all patients plus several individual files per patient allows for an overall analysis as well as the possibility to look up precise individual patient data when needed.

2.4 Statistics

R statistical software (version 4.0.3; The R Foundation for Statistical Computing, Vienna, Austria) was used for all statistical calculations. Median, range, and percentages were used for descriptions of nominal and ordinal data as indicated. The Kaplan-Meier method and log-rank test were applied for comparisons of overall survival. A univariate and multivariate Cox-regression model was used in order to determine possible prognostic factors of overall survival. A dichotomization of continuous covariates, such as laboratory values, for example based on the median value of the population, was not conducted. In the univariate Cox-regression model p-values < 0.1 were regarded as statistically significant and the respective covariates were included in the multivariate model. The assumption of proportional hazards was tested for the respective covariates. Regarding the univariate analysis, all covariates but platelet count fulfilled the proportional hazard assumption.

With regard to the prognostic significance of low platelet counts in previous publications (see chapter 1.7.2), and in order to be able to represent the three main blood lineages, this covariate was not excluded from the calculations. [2]

For initial laboratory findings, the data which was obtained on the day of the initiation of a specific HLH-directed therapy were used. When no data was available for this day, primarily the first value up to 72 hours before or if lacking, 72 hours after beginning of treatment, was used. In case that there existed more than one value for a specific laboratory parameter per day, the value that was measured within the first blood sample of the morning (usually between 5 – 9 am) was used for the calculations. If a laboratory value was not measured exactly above or below a specific cutoff point, it was modified by the addition or subtraction of one point to the last decimal place (e.g., for ferritin values > 40,000 µg/L the value 40,001 µg/L and for leukocytes < 0.1 /nL the value 0.09 /nL was noted). Regarding calculation on overall survival, the timespan from the initiation of HLH-specific therapy until appearance of death of any cause or the date of the last-follow-up up to the end of the study period at 01/31/2021 was used. [2]

All plots were created with R's *ggplot2* function and Kaplan-Meier survival curves with the *ggsurvplot* function. P-values < 0.05 were regarded as statistically significant.

3. Results

3.1 Patient overview

Forty individuals were male (40/62, 65%) and the median age at diagnosis was 53.5 years (range, 19-81 years). The median follow-up of the surviving individuals reached 288 days (range, 3-2611 days). Most patients were treated in the year 2020 (17/62, 27%). An overview of the number of treated patients per year and hospital is shown in Figure 11. The median time from hospital admission to initial HLH-directed therapy was 10.5 days (range, 0-67 days). The median hospital stay was 43 days (range, 6-127 days). Surviving patients had a median hospital stay of 49 days (range, 6-98 days) and deceased patients had a median hospital stay of 37.5 days (range 7-127 days). Thirty-five patients (35/62, 56%) needed treatment in an ICU with a median ICU stay of eight days (range, 1-78 days). Twenty-three patients (23/62, 37%) were in need of respiratory support, and 19 (19/62, 31%) needed hemodialysis treatment. One or more recurrent HLH episodes appeared in 10 patients (10/62, 16%). [2]

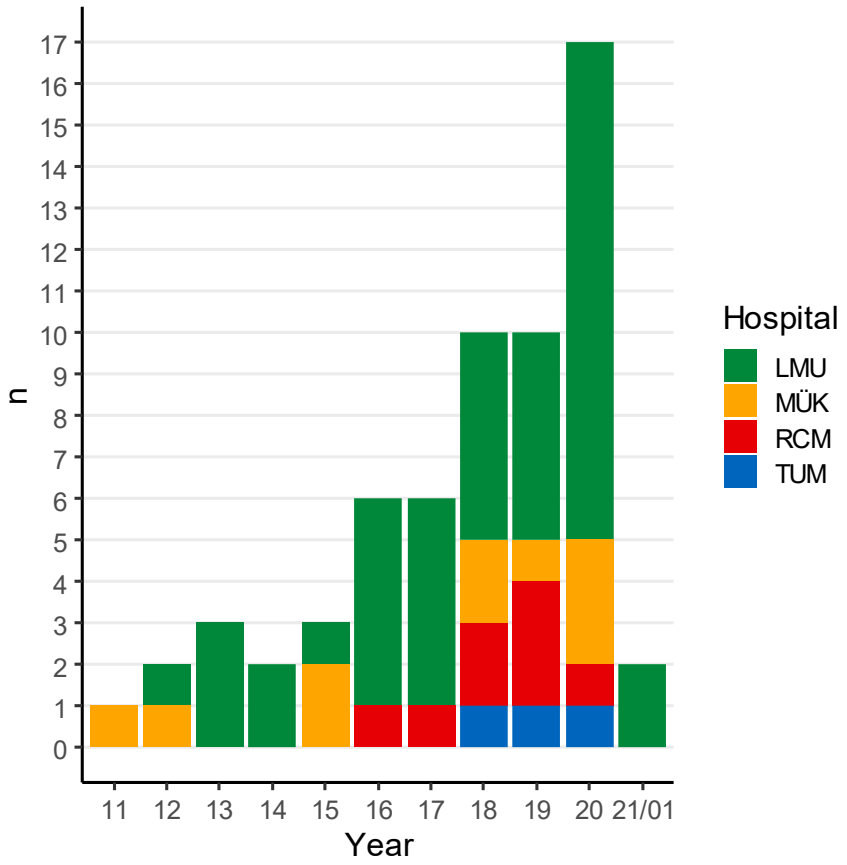

Figure 11 HLH cases treated per hospital and year. *LMU*, University Hospital LMU Munich; *MÜK*, Munich Clinic Schwabing; *RCM,* Red Cross Hospital Munich; *TUM*, Klinikum rechts der Isar, Technical University of Munich (TUM). (Wimmer et al., *sCD25 as an independent adverse prognostic factor in adult patients with HLH: results of a multicenter retrospective study* (supplement) © 2023 by Elsevier [2], permission of the publisher as author of the article not required).

The population was divided into four etiologic subgroups according to the most likely trigger of the HLH episode: malignancy, infection, autoimmune disease, and idiopathic. An overview of the etiologies per hospital is shown in Figure 12. The exact triggers are depicted in Table 3. An overview of the standard patient data per etiology is shown in Table 4.

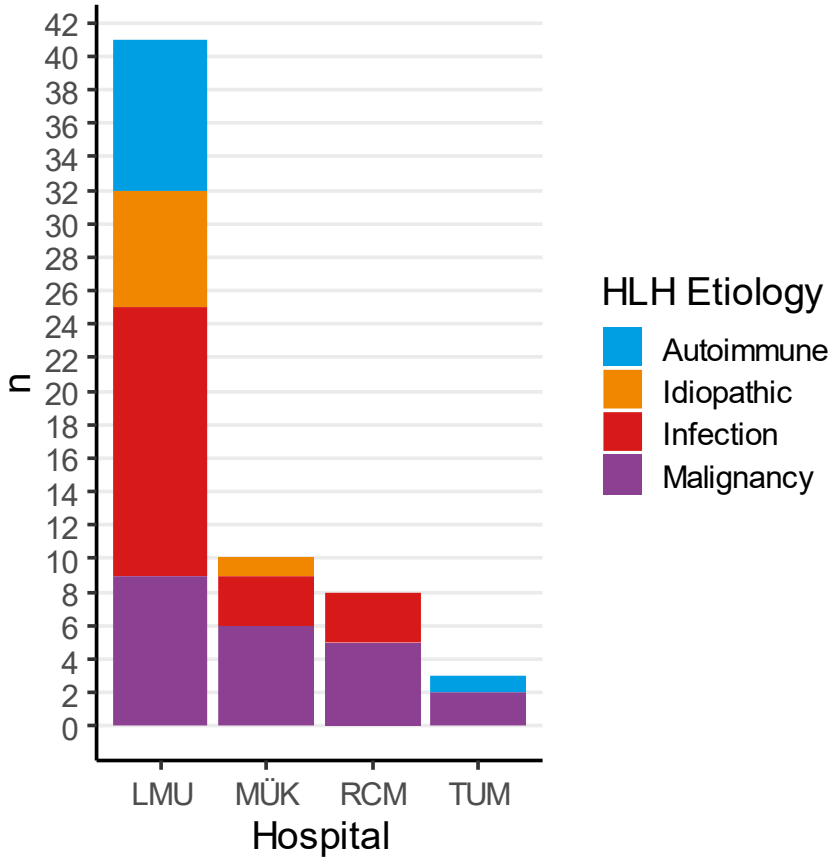

Figure 12 Etiologies per hospital. *LMU*, University Hospital LMU Munich; *MÜK*, Munich Clinic Schwabing; *RCM,* Red Cross Hospital Munich; *TUM*, Klinikum rechts der Isar, Technical University of Munich (TUM).

Table 3 HLH etiologies of the population (Wimmer et al., *sCD25 as an independent adverse prognostic factor in adult patients with HLH: results of a multicenter retrospective study* © 2023 Elsevier [2], permission of the publisher as author of the article not required).

Etiology	n	(%)	Comment
Malignancies	**22**	**(35)**	
Myeloid	2	(3)	
AML	1	(2)	
MDS	1	(2)	
B-lymphoid	11	(18)	
Hodgkin lymphoma	4	(6)	EBV-association n = 4
DLBCL	3	(5)	EBV-association n = 2
B-cell lymphoma	1	(2)	
Primarily cerebral B-cell lymphoma	1	(2)	
Multiple myeloma	2	(3)	after autologous SCT n = 2
T/NK-lymphoid	8	(13)	
T/NK-cell lymphoma	2	(3)	
PTCL-NOS	2	(3)	
Angioimmunoblastic T-cell lymphoma	1	(2)	EBV-association
Mycosis fungoides	1	(2)	
NK cell leukemia	1	(2)	EBV-association
T-cell-neoplasia (not further classified)	1	(2)	
Clinical suspicion of lymphoma	1	(2)	
Infections	**22**	**(35)**	
Viral	16	(26)	
EBV	12	(19)	after SCT n = 1
CMV	3	(5)	after SCT n = 1
HHV-6	1	(2)	
Bacteria	3	(5)	
Escherichia coli	1	(2)	
Streptococcus agalactiae	1	(2)	
Streptococcus pyogenes	1	(2)	
Fungal	2	(3)	
Aspergillus fumigatus	1	(2)	viral coinfection suspected
Candida glabrata	1	(2)	after urosepsis (*Klebsiella spp.*)
Unknown/multiple	1	(2)	
Autoimmune/systemic	**10**	**(16)**	
AOSD	5	(8)	
Antisynthetase syndrome	2	(3)	
Hypereosinophilic syndrome	1	(2)	
Immune complex vasculitis	1	(2)	
Sweet-Syndrome	1	(2)	
Idiopathic	**8**	**(13)**	

AML, acute myelogenous leukemia; *AOSD*, Adult-onset Still's disease; *CMV*, Cytomegalovirus; *DLBCL,* diffuse large B-cell lymphoma; *EBV*, Epstein-Barr virus; *HHV-6*,

Human herpesvirus 6; *MDS*, myelodysplastic syndrome; *PTCL-NOS*, Peripheral T-Cell lymphoma not otherwise specified; *SCT,* stem cell transplantation.

3.1.1 Malignant subgroup (M-HLH)

Hematologic malignancies were the most probable trigger of the HLH episode in 22 patients (22/62, 35%). Fifteen of these (15/22, 68%) were male and the median age at diagnosis was 60.5 years (range, 24-81 years).

The initial diagnosis of the underlying malignancy was made during the HLH related hospital stay in 13 cases (13/22, 59%). In seven of these, HLH was diagnosed before the confirmation of the underlying malignancy, with a median timespan between the respective diagnoses of five days (range, 2-45 days) [2]. The presence of malignancy was already known in the other nine individuals (9/22, 41%): HLH occurred either within a progress (Mycosis fungoides), during chronic active disease (diffuse large B-cell lymphoma (DLBCL), EBV-associated Hodgkin lymphoma), after application of chemotherapies (2x DLBCL with EBV-association, primarily cerebral B-cell lymphoma, AML) or after alloSCT (2x multiple myeloma).

Of note, two patients developed HLH due to a viral trigger (CMV pneumonia, EBV reactivation, respectively) after SCT (autologous SCT in late recurrence of Hodgkin lymphoma, alloSCT in high-risk pre-T acute lymphoblastic leukemia) and were therefore included in the infectious subgroup in allusion to Lehmberg et al. [66]. [2]

The majority of malignancies were either of B-lymphoid (11/22, 50%) or T/NK-lymphoid (8/22, 36%) origin. A lymphoma of unknown origin was clinically suspected in a 72-year-old female with splenomegaly and a combined retrosternal, infraclavicular, mediastinal, and retroperitoneal lymphadenopathy. Despite the lacking histopathologic proof of a malignancy, she was included in the malignant subgroup in accordance with the opinion of the treating physicians mentioned in the patient files.

Eight malignancies (8/22, 36%) showed an EBV association, among them all four cases of HLH triggered by Hodgkin lymphoma.

Median survival in the malignant subgroup was 48 days and one-year survival probability reached 21%. Overall mortality rate was 17/22 (77%).

3.1.2 Infectious subgroup (I-HLH)

Twenty-two patients (22/62, 35%) had an infectious triggered HLH episode. Sixteen individuals (16/22, 73%) were male and the median age at diagnosis was 47.5 years (range, 19-81 years).

The majority of cases was affiliated with viral infections, in particular with EBV, which was the responsible trigger in 12 patients (12/22, 55%). Further virologic triggers were CMV, present in three (3/22, 14%), and HHV-6 in one patient (1/22, 5%), respectively. Bacterial infection as most likely HLH trigger was present in three patients (3/22, 14%): *Escherichia coli* (smear sample of infected fistula, *XIAP* deficiency), *Streptococcus agalactiae* (blood culture), and *Streptococcus pyogenes* (blood culture, suspected HSV-coinfection). Fungal antigens were detected around onset of HLH disease in two individuals (2/22, 9%): *Aspergillus fumigatus* (blood culture + serology, virologic coinfection suspected) and *Candida glabrata* (urine, after urosepsis with *Klebsiella spp.*). One patient (1/22, 5%) had qualitative proof of multiple viral DNA (EBV, Parvovirus B19, HSV), plus positive tests in aspergillus antigen ELISA, without the identification of a predominant HLH trigger, and was therefore labeled as carrying an *unknown infectious* trigger.

Median survival in the infectious subgroup was 839 days and one-year survival probability reached 62%. Overall mortality rate was 10/22 (45%).

3.1.3 Autoimmune subgroup (MAS-HLH)

HLH developed either because of an autoimmune, autoinflammatory or systemic disease in 10 patients (10/62, 16%) (MAS-HLH). Four individuals were male (4/10, 40%) and the median age at diagnosis was 52.5 years (range, 21-72 years).

The most common entity was AOSD, present in five patients (5/10, 50%). Two individuals (2/10, 20%) suffered from antisynthetase-syndrome and three patients either had an immunocomplex-vasculitis (1/10, 10%), or a hyper-eosinophilic-syndrome (1/10, 10%), or a Sweet-syndrome (1/10, 10%), respectively.

One-year survival probability within the MAS-HLH group was 90% and median survival was not reached. Overall mortality rate was 2/10 (20%).

3.1.4 Idiopathic subgroup

No specific trigger of the HLH episode could be identified in eight patients (8/62, 13%). Five of these individuals were male (5/8, 63%) and the median age at diagnosis was 57 years (range, 29-72 years).

Median survival in the idiopathic subgroup was 65 days and one-year survival probability reached 46%. Overall mortality rate was 3/8 (38%).

Table 4 Overview of epidemiologic data per etiology

	Total	**Malig-nancy**	**Infection**	**Autoim-mune**	**Idiopathic**
Age median (range)	53.5 (19-81)	60.5 (24-81)	47.5 (19-81)	52.5 (21-72)	57 (28-74)
Male sex (%)	40/62 (65%)	15/22 (68%)	16/22 (73%)	4/10 (40%)	5/8 (63%)
Median survival [days] (95% CI)	288 (72-NA)	48 (28-NA)	839 (82-NA)	Not reached (1187-NA)	65 (45-NA)
One-year survival probability (95% CI)	49.5% (38-65%)	21% (8-51%)	62% (44.5-87%)	90% (73-100%)	46% (17.5-100%)
Overall mortality (%)	32/62 (52%)	17/22 (77%)	10/22 (45%)	2/10 (20%)	3/8 (38%)

CI, Confidence interval.

3.2 Diagnostic criteria

All single fulfilled diagnostic parameters of the HLH-2004 criteria and the HScore for each patient are shown in two separate heat maps (Figure 13, Figure 14).

An overview of the respective diagnostic parameters of the HLH-2004 criteria and HScore of the population is shown in Table 5.

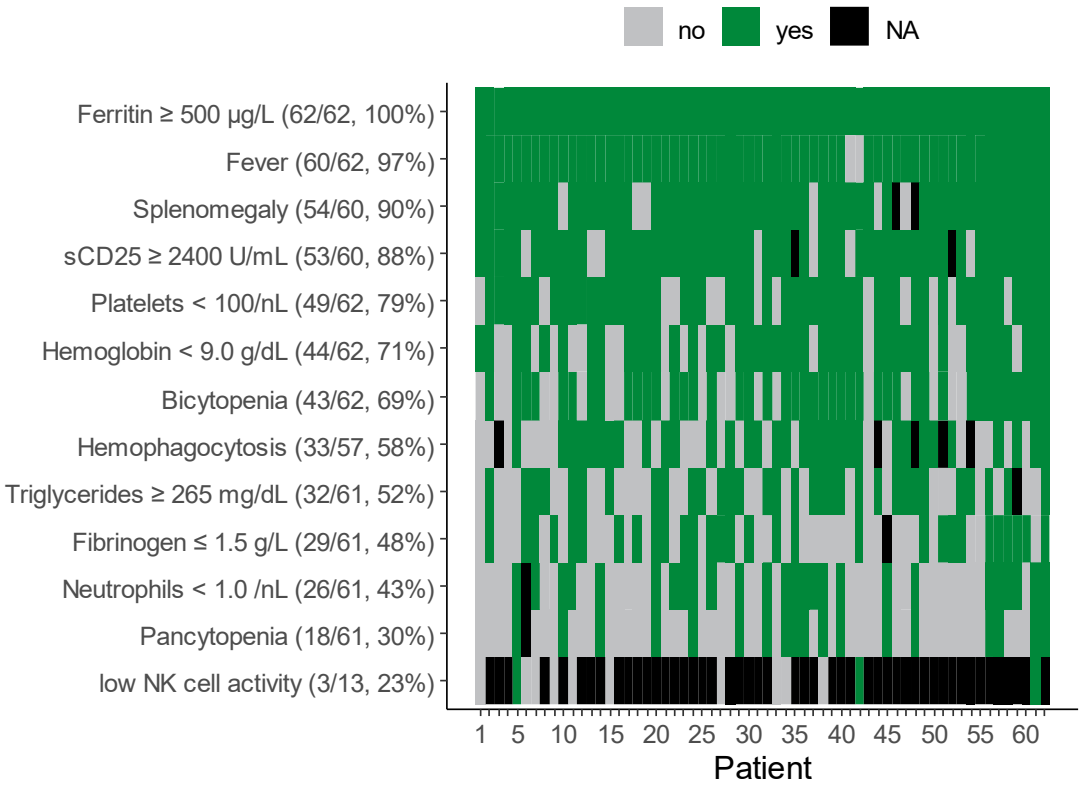

Figure 13 Heat map of HLH-2004 criteria per patient. *NK cell*, Natural killer cell; *sCD25*, alpha chain of the soluble interleukin-2 receptor.

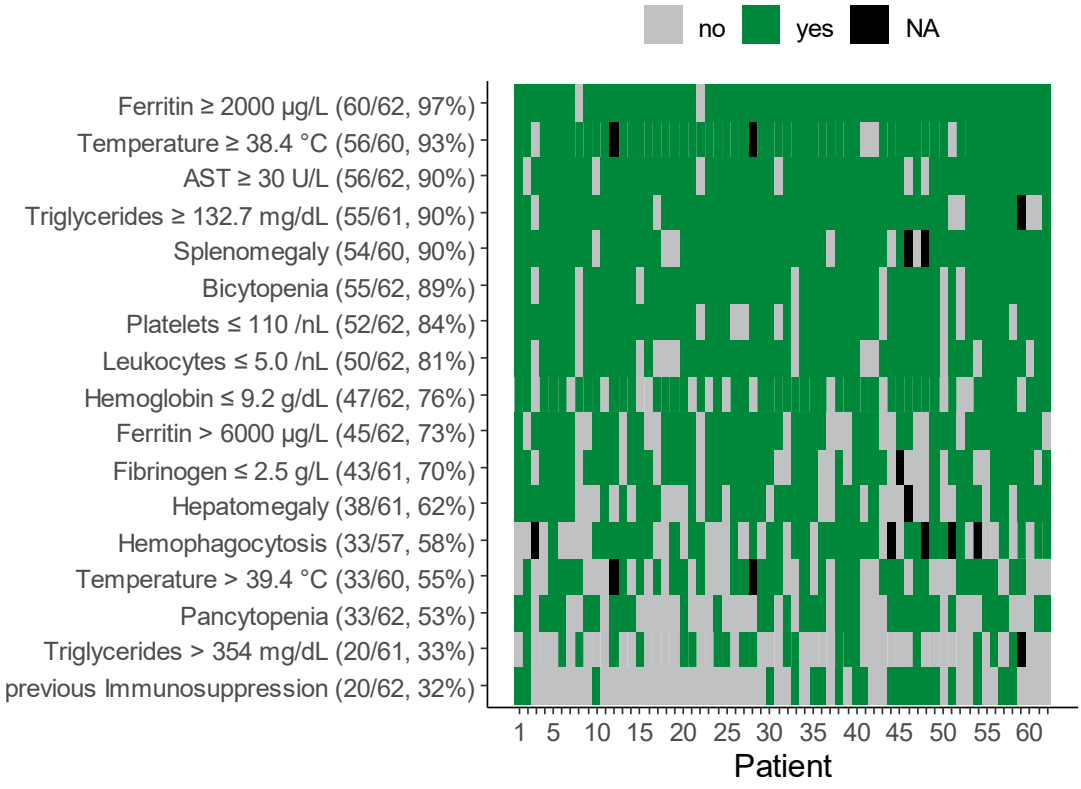

Figure 14 Heat map of HScore criteria per patient. *AST*, Aspartate transaminase.

Table 5 Diagnostic criteria per etiologic subgroup (Wimmer et al., *sCD25 as an independent adverse prognostic factor in adult patients with HLH: results of a multicenter retrospective study* © 2023 by Elsevier [2], permission of the publisher as author of the article not required).

	Total			Malignancy			Infection			Autoimmune			Idiopathic		
Criterium	N	Total	(%)	N	Total	(%)	N	Total	(%)	N	Total	(%)	N	Total	(%)
Ferritin															
Ferritin ≥ 500 µg/L	62	62	(100)	22	22	(100)	22	22	(100)	10	10	(100)	8	8	(100)
Ferritin ≥ 2000 µg/L	60	62	(97)	22	22	(100)	22	22	(100)	8	10	(80)	8	8	(100)
Ferritin > 6000 µg/L	45	62	(73)	17	22	(77)	16	22	(73)	7	10	(70)	5	8	(63)
Temperature															
Fever	60	62	(97)	22	22	(100)	21	22	(95)	10	10	(100)	7	8	(88)
Temperature ≥ 38.4 °C	56	60	(93)	20	21	(95)	19	21	(90)	10	10	(100)	7	8	(88)
Temperature > 39.4 °C	33	60	(55)	10	21	(48)	13	21	(62)	5	10	(50)	5	8	(63)
Organomegaly															
Splenomegaly	54	60	(90)	19	20	(95)	18	22	(82)	10	10	(100)	7	8	(88)
Hepatomegaly	38	61	(62)	11	21	(52)	15	22	(68)	7	10	(70)	5	8	(63)
Hepatosplenomegaly	38	61	(62)	11	21	(52)	15	22	(68)	7	10	(70)	5	8	(63)
Hemoglobin															
Hemoglobin < 9.0 g/dL	44	62	(71)	17	22	(77)	14	22	(64)	8	10	(80)	5	8	(63)
Hemoglobin ≤ 9.2 g/dL	47	62	(76)	20	22	(91)	14	22	(64)	8	10	(80)	5	8	(63)
Platelets															
Platelets < 100 /nL	49	62	(79)	19	22	(86)	19	22	(86)	4	10	(40)	7	8	(88)
Platelets ≤ 110 /nL	52	62	(84)	20	22	(91)	19	22	(86)	6	10	(60)	7	8	(88)
White Blood Cells															
Neutrophils < 1.0 /nL	26	61	(43)	10	22	(45)	8	21	(38)	5	10	(50)	3	8	(38)
Leukocytes ≤ 5.0 /nL	50	62	(81)	19	22	(86)	17	22	(77)	9	10	(90)	5	8	(63)
Cytopenia															
Bicytopenia [HLH-04]	43	62	(69)	17	22	(77)	14	22	(64)	5	10	(50)	7	8	(88)
Bicytopenia [HScore]	55	62	(89)	21	22	(95)	18	22	(82)	9	10	(90)	7	8	(88)
Pancytopenia [HLH-04]	18	61	(30)	8	22	(36)	7	21	(33)	3	10	(30)	0	8	(0)
Pancytopenia [HScore]	33	62	(53)	17	22	(77)	10	22	(45)	4	10	(40)	2	8	(25)
sCD25 ≥ 2400 U/mL	53	60	(88)	20	21	(95)	20	21	(95)	8	10	(80)	5	8	(63)
Histopathological hemophagocytosis	33	57	(58)	7	19	(37)	12	20	(60)	8	10	(80)	6	8	(75)
Triglycerides															
TGs ≥ 132.7 mg/dL	55	61	(90)	20	22	(91)	20	22	(91)	9	10	(90)	6	7	(86)
TGs ≥ 265 mg/dL	32	61	(52)	15	22	(68)	11	22	(50)	3	10	(30)	3	7	(43)
TGs > 354 mg/dL	20	61	(33)	8	22	(36)	8	22	(36)	3	10	(30)	1	7	(14)
Fibrinogen															
Fibrinogen ≤ 1.5 g/L	29	61	(48)	13	21	(62)	9	22	(41)	3	10	(30)	4	8	(50)
Fibrinogen ≤ 2.5 g/L	43	61	(70)	15	21	(71)	17	22	(77)	6	10	(60)	5	8	(63)
TGs ≥ 265 mg/dL or Fibrinogen < 1.5 g/L	43	61	(70)	18	21	(86)	15	22	(68)	5	10	(50)	5	8	(63)
Low/absent NK cell activity	3	13	(23)	0	2	(0)	2	7	(29)	1	2	(50)	0	2	(0)
AST ≥ 30 U/L	56	62	(90)	19	22	(86)	21	22	(95)	8	10	(80)	8	8	(100)
Previous immunosuppression	20	62	(32)	10	22	(45)	5	22	(23)	2	10	(20)	3	8	(38)

AST, aspartate transaminase; *NK cell*, natural killer cell; *sCD25*, alpha chain of the soluble interleukin-2 receptor; *TG*, triglycerides.

3.2.1 Ferritin

Each patient of the study population (62/62, 100%) presented with ferritin values > 500 µg/L. Most of the individuals also exceeded the increased cutoff points of the HScore at 2,000 (60/62, 97%) and 6,000 µg/L (45/62, 73%), respectively. The median ferritin level was 11,832 µg/L (range, 701-232,000 µg/L). [2] An overview of the ferritin and sCD25 values per patient depicted by outcome is shown in Figure 15.

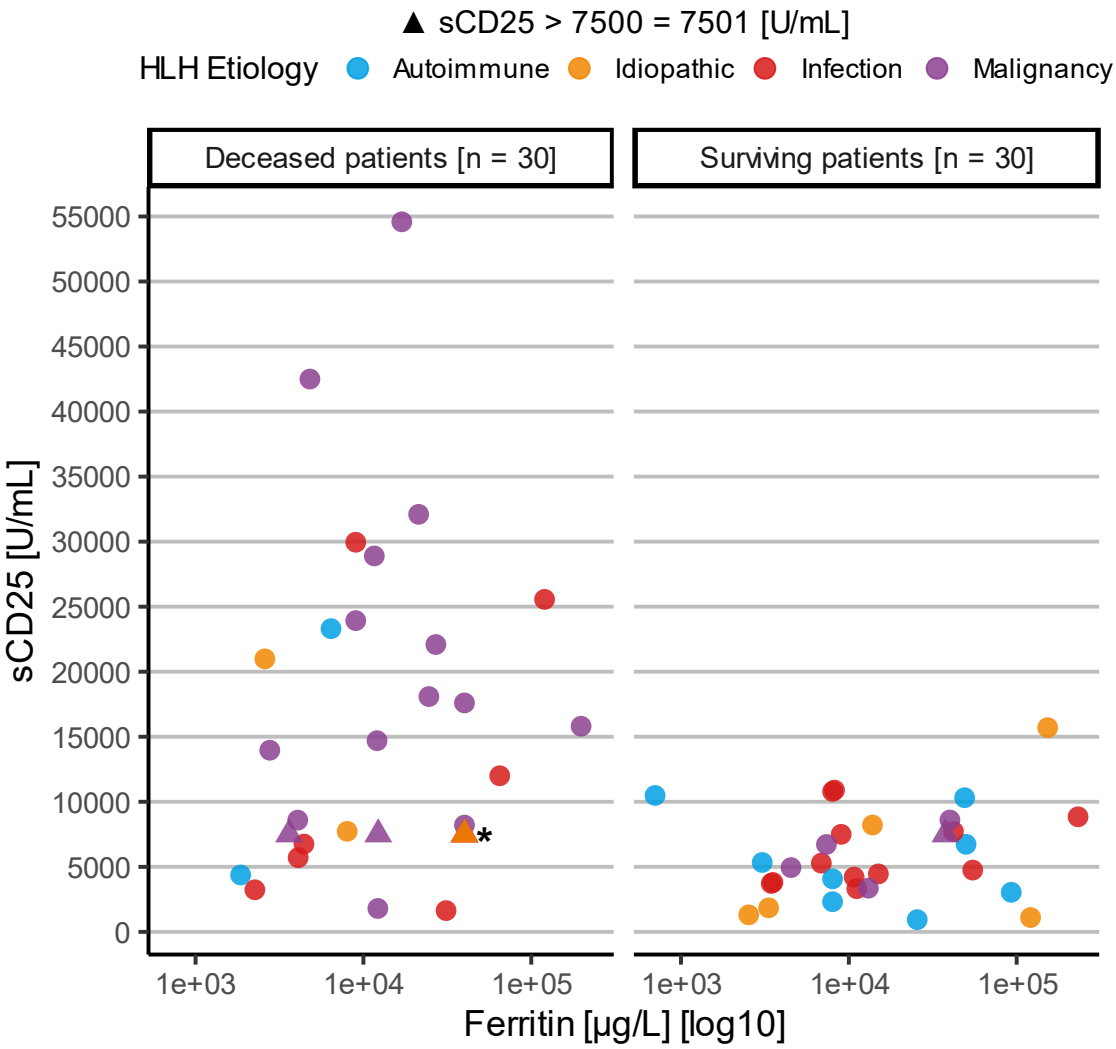

Figure 15 sCD25 and ferritin values of the population according to outcome. The underlying HLH etiology is depicted by different colors.

* overlap of three patients (etiologies: 2x infection, 1x idiopathic) with sCD25 levels of 7,501 U/mL and ferritin values of 40,001 µg/L. (Wimmer et al., *sCD25 as an independent adverse prognostic factor in adult patients with HLH: results of a multicenter*

retrospective study © 2023 by Elsevier [2], permission of the publisher as author of the article not required)

3.2.2 Fever, hepatosplenomegaly and cytopenia

A majority of 55 patients (55/62, 89%) simultaneously presented with all three symptoms of the clinical HLH triad marked by fever, splenomegaly, and bi-/pancytopenia [61]. The presence of fever was described in 60 individuals (60/62, 97%). Fifty-six (56/60, 93%) patients had documented temperatures ≥ 38.4 °C, with 33 (33/60, 55%) reaching a maximum temperature higher than 39.4 °C. The median temperature was 38.95 °C (range, 37.7-41 °C). Presence of splenomegaly was described in 54 patients (54/60, 90%), who had undergone adequate imaging procedures. An additional hepatomegaly was detected in 38 patients (38/61, 62%). [2]

The requirements for cytopenia differ between the HLH-2004 criteria and the HScore: the HScore is using leukocytes instead of neutrophil granulocytes and is having higher cutoffs for anemia (hemoglobin < 9.2 g/dL instead of < 9.0 g/dL) and thrombocytopenia (platelet counts ≤ 110 /nL instead of < 100 /nL). Therefore, 55 patients (55/62, 89%) met the criteria of a bicytopenia in the HScore, compared to 43 individuals (43/62, 69%) when applying the HLH-2004 criteria. [2]

Median levels of the hematologic parameters were: hemoglobin 7.85 g/dL (range, 4.9-14.4 g/dL), platelets 42 /nL (range, 1-193 /nL), leucocytes 1.67 /nL (range, 0.03-60.64 /nL), and neutrophil granulocytes 1.19 (range, 0.04-51.2 /nL).

3.2.3 sCD25

At least one sCD25 value was available for 60 (60/62, 97%) patients. Fifty-three (53/60, 88%) had values above 2400 U/mL and therefore met the corresponding HLH-2004 criterion. The median sCD25 value of the study population was 7501 U/mL (range, 939-54589 U/mL). An overview of the course of all sCD25 values per patient depicted by outcome is shown in Figure 16. [2]

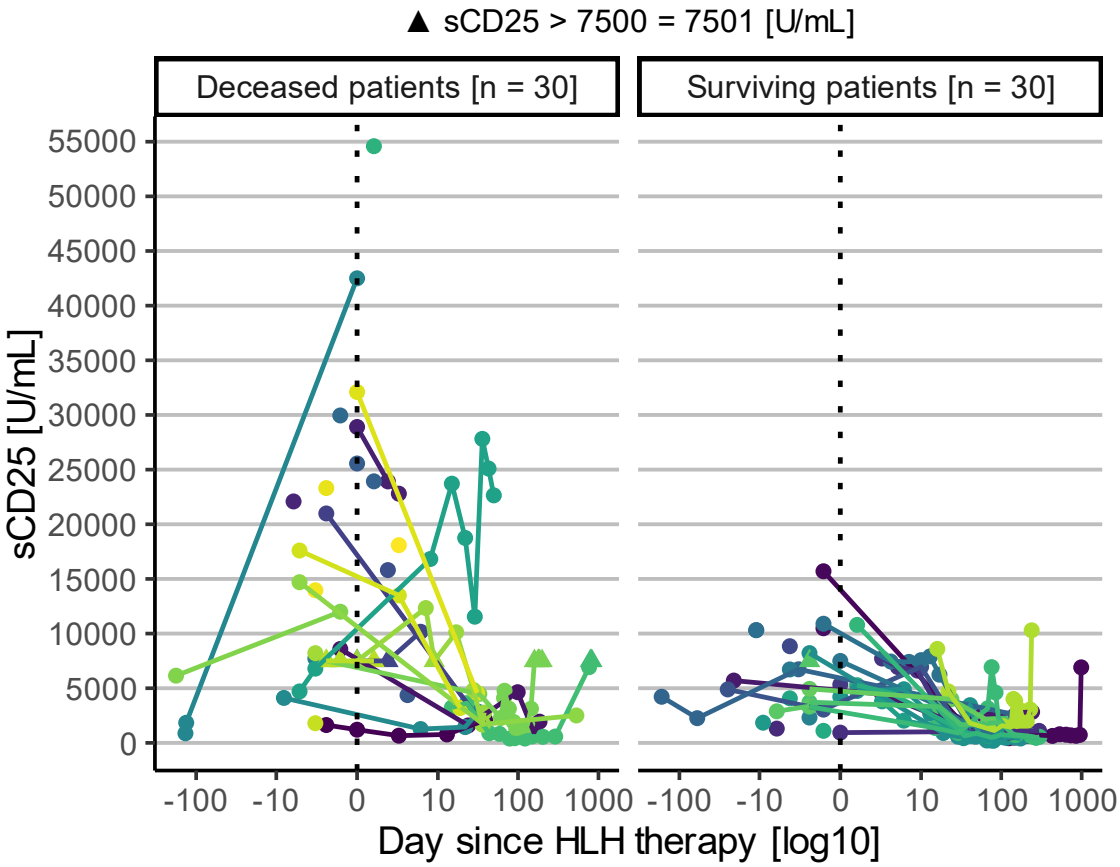

Figure 16 sCD25 trends per patient and outcome. No patient with a maximum sCD25 value > 15,701 U/mL survived within the study period. *sCD25*, alpha chain of the soluble interleukin-2 receptor. (Wimmer et al., *sCD25 as an independent adverse prognostic factor in adult patients with HLH: results of a multicenter retrospective study* (supplement) © 2023 by Elsevier [2], permission of the publisher as author of the article not required).

3.2.4 Proof of hemophagocytosis

Fifty-seven patients (57/62, 92%) received one or more bone marrow examinations during their first HLH-associated hospital stay. Signs of active hemophagocytosis was detected in 33 individuals (33/57, 58%). In the majority of 30 patients (30/33, 91%), proof of hemophagocytosis could be detected in the initial analysis. Two individuals did not present with hemophagocytosis in the initial, but following examination. Temporal distance between these two examinations was 16 and 29 days, respectively. In one patient, who had undergone two bone marrow examinations within an interval of 17 days, signs of hemophagocytosis in bone marrow and splenic tissue was not found until post mortem obduction (34 days after the second bone marrow analysis). Hemophagocytosis in splenic

tissue was also detected in two female patients: one had undergone splenectomy due to suspicion of lymphoma prior to developing HLH while the other one underwent post mortem obduction after suffering from HLH associated with DLBCL.

In the majority of 39 patients (39/57, 68%) a bone marrow biopsy as well as an aspiration was performed. In addition, each nine patients received either only a biopsy (9/57, 16%) or only an aspiration (9/57, 16%). Regarding the 23 patients with a proven hemophagocytosis where a biopsy and an aspiration was performed simultaneously, the presence of hemophagocytosis was solely detected in the aspiration sample in 10 patients (10/23, 43%), solely in the biopsy sample in eight patients (8/23, 35%), and in the aspiration as well as in the biopsy sample in the remaining five cases (5/23, 22%). [2] An example of positive findings of hemophagocytosis in the cytomorphology of a bone marrow aspirate is shown in Figure 17.

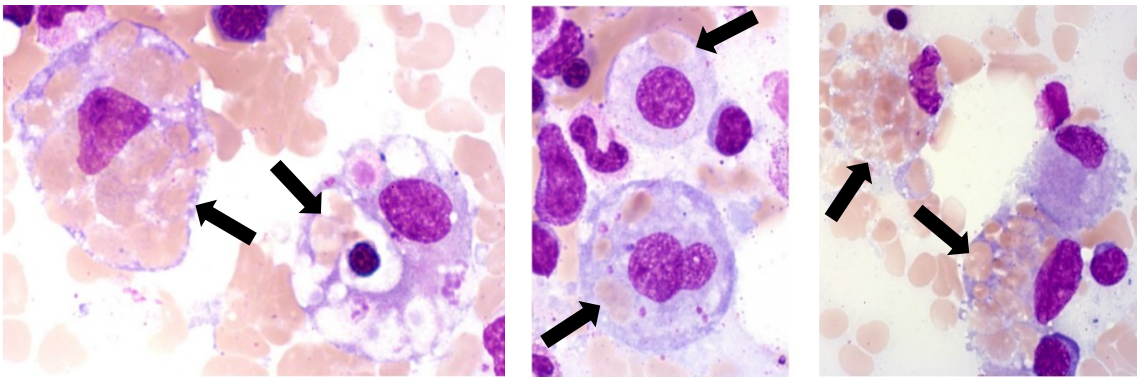

Figure 17 Examples of hemophagocytosis in bone marrow aspirate (Laboratory for leukemia diagnostic, University Hospital LMU Munich)

3.2.5 Triglycerides and fibrinogen

Forty-three patients (43/61, 70%) had either a hypertriglyceridemia or hypofibrinogenemia according to the HLH-2004 criteria: 32/61 (52%) had triglyceride levels ≥ 265 mg/dL and 29/61 (48%) presented a hypofibrinogenemia of < 1.5 g/L. Concerning triglycerides, 50/61 (82%) surpassed the lower and 20/61 (33%) the higher cutoff point of the HScore with values ≥ 132.7 mg/dl and ≥ 354 mg/dl, respectively. Fibrinogen levels ≤ 2.50 g/L were present in 43/61 (70%) patients.

The median level of triglycerides was 295 mg/dL (range, 89-718 mg/dl) and 163 mg/dL (range, 34-842 mg/dL) for fibrinogen.

3.2.6 Natural killer cell activity

NK cell activity tests were conducted in the samples of 16 patients (16/62, 26%). However, due to severe NK cell depletion, NK cell activity could only be analyzed in 13 cases. Three samples (3/13, 23%) showed a reduced NK cell activity: in two of them both a decreased spontaneous NK cell degranulation as well as a decreased IL-2 stimulated NK cell degranulation was found; regarding the third sample, a decreased NK cell activity was described in the patient files without the availability of result specific documents. [2]

One sample presented pathologic results in spontaneous NK cell degranulation which disappeared after stimulation with IL-2 and was therefore considered as non-pathologic, as suggested by Bryceson et al. [157].

3.2.7 Genetics

Six patients (6/62, 10%) received HLH specific genetic testing. A 51-year-old male with idiopathic HLH and a 35-year-old female with consanguine parents (cousins) and EBV-triggered HLH, were diagnosed with homozygous mutations in the perforin encoding *PRF1* gene (FHL2). The male patient presented a homozygous c.272C>T; p.Ala91Val variant and the woman a homozygous c.208G>T; p.Asp70Tyr mutation. In a 42-year-old male with reduced NK cell activity, consanguine parents (cousins), and EBV-triggered HLH, a heterozygous variant of unknown significance was found both in the FHL3 associated *UNC13D* gene (c.175G>A; p.ala59Thr) and in the FHL5 associated *STXP2* (c.1298C>T; p.Ala433Val) gene. [2]

Five patients of male sex underwent testing for mutations in the *XIAP* gene. Two within the aforementioned HLH gene panels and three of them after presenting a reduced *XIAP* expression in their NK cells. A hemizygous variant of the *XIAP* gene could be identified in two patients with a reduced *XIAP* expression. One was a 19-year-old male presenting with the c.978_1099del; p.Cys327Ter variant, and the other one was a 42-year-old male with the c.1141C>T; p.Arg381* variant. [2]

3.2.8 Aspartate transaminase

A majority of 90% (56/62) presented with AST values ≥ 30 U/L. The median AST level of the population was 207 U/L (range, 18-8635 U/L).

3.2.9 Previous immunosuppression and activation

An immunosuppression prior to the manifestation of the HLH episode was present in 20 patients (20/62, 32%). The most common entity were chemotherapies, which were administered to six patients (6/20, 30%) up to 90 days before the manifestation of the HLH episode. Five patients (5/20, 25%) received immunosuppressive agents because of autoimmune diseases, four patients underwent SCT (4/20, 20%), and four individuals were under glucocorticoid treatment (4/20, 20%). One patient (1/20, 5%) developed HLH 7.5 years after a renal transplantation and accompanying cyclosporine therapy.

A 70-year-old male patient with residual T-cell-rich large B-cell lymphoma received a combination chemotherapy with fludarabine and cyclophosphamide plus additional immune activating CART two weeks prior to developing HLH. Despite not being part of the diagnostic criteria, previous publications described the possibility of developing HLH due to these novel T-cell engaging biological therapies [88, 89].

3.2.10 Further symptoms

Hydroptic symptoms were present in 55 patients (55/62, 89%). Most common were pleural effusions (49/55, 89%), ascites (31/55, 56%), and pericardial effusions (18/55, 33%).

Forty individuals had a lymphadenopathy (40/62, 65%).

Neurologic symptoms (excluding headache, n = 11) during the HLH episode were present in 35 individuals (35/62, 56%): most frequent were reduced vigilance (17/35, 49%) and confusion (13/35, 37%). Delirium occurred in seven (7/35, 20%) individuals, and five patients (5/35, 14%) with different etiologies (2x malignancy, 2x autoimmune, 1x infection) had a seizure.

Thirty-four individuals (34/62, 55%) presented with hepatic abnormalities, foremost with steatosis hepatis (21/34, 62%) and icterus (14/34, 41%).

Dermatological symptoms were present in 26 individuals (26/62, 42%): most common were exanthems (19/26, 73%) and erythema (6/26, 23%).

Twenty-six patients (26/62, 42%) had gastrointestinal symptoms: most common were diarrhea (18/26, 69%), nausea (13/26, 50%), and vomiting (9/26, 35%).

3.3 Complications

During the course of HLH, 27 patients (27/62, 44%) developed a pneumonia; among them were eight cases (8/27, 30%) of fungal pneumonia. Liver damage/failure occurred in 23 (23/62, 37%), and renal failure in 22 (22/62, 35%) patients, respectively. Coagulation disorders appeared in 21 (21/62, 34%) patients. According to the documents, 15 of them (15/21, 71%) developed a disseminated intravascular coagulopathy (DIC). Sepsis appeared in 18 patients (18/62, 29%) and nine individuals (9/62, 15%) developed a septic shock. Bleeding events took place in 14 (14/62, 23%) patients. Most common were gastroenteric bleedings (6/14, 43%). Thromboembolic events were described in 11 patients (11/62, 18%); most frequent were splenic infarctions (9/11, 82%). Further complications were newly occurring atrial fibrillations present in nine (9/62, 15%), and a systemic mycosis in six (6/62, 10%) patients, respectively.

3.4 Metabolic imaging

One aim of this study was to evaluate the usage of metabolic imaging in adult HLH patients. Therefore, the results of 26 patients (26/62, 42%) who received metabolic imaging procedures were reviewed and analyzed. Twenty-five of these underwent PET-CT imaging and in one individual full-body scintigraphy was performed. [2]

The most common findings were abnormalities in the lymph nodes and skeleton: either a generalized lymphadenopathy or an increased standardized uptake value (SUV) in the lymph nodes with a median SUVmax of 5.7 (range 2.3-24) could be detected in 19 patients (19/26, 73%). Seventeen patients (17/26, 65%) presented with a general increase in the SUV of the skeleton/bone marrow. A further common finding was the presence of a hypermetabolic splenomegaly, which was described in 10 patients (10/25, 40%). [2]

Subsequent biopsies in suspicious tissues were performed in eight patients (8/26, 31%). However, no signs of either hemophagocytosis or malignancy were found within them.

An exemplary PET-CT image of a 19-year-old male suffering from EBV-triggered HLH accompanied by a massive hepatosplenomegaly with kissing phenomenon and hypermetabolic splenomegaly, in addition to an increased uptake of 2-deoxy-2-[18F]fluoro-D-glucose (^{18}F-FDG) in several lymph nodes is shown in Figure 18.

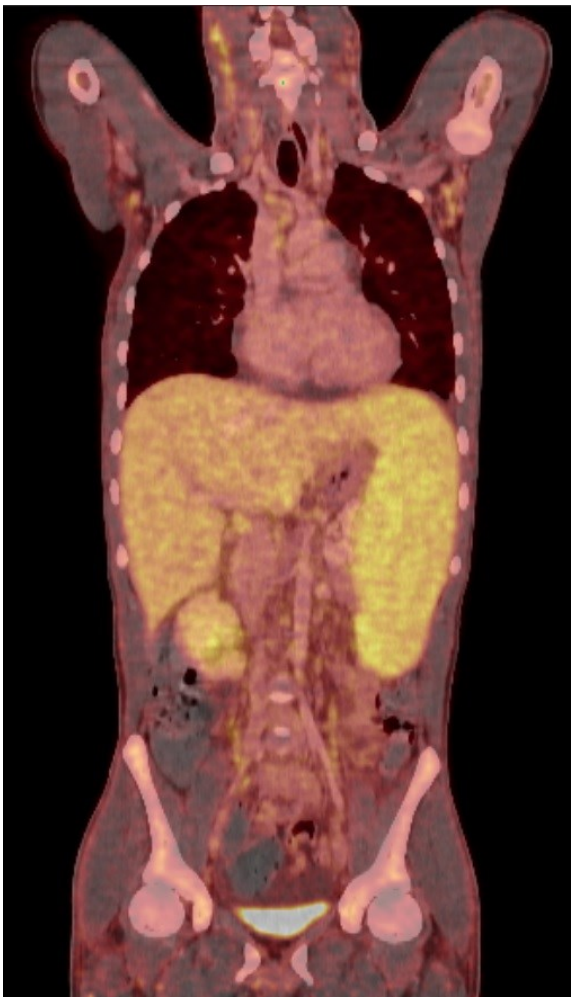

Figure 18 PET-CT of a 19-year-old male with EBV-triggered HLH and *XIAP*-deficiency: presence of hepatosplenomegaly with kissing phenomenon and hypermetabolic splenomegaly (SUVmax = 5.1), and increased ^{18}F-FDG uptake in selected enlarged lymph node stations (cervical (SUVmax = 5.1), axillar (SUVmax = 2.4), hilar (SUVmax = 3.9), mediastinal, intraabdominal, retroperitoneal (SUVmax = 1.7)). (Departments of Nuclear Medicine and Radiology, LMU Munich)

3.5 Initial therapies

An overview of the agents used for initial HLH treatment is shown in Table 6, and a heat map depicting the individual agents per patient is shown in Figure 19.

Table 6 HLH-directed therapies (Wimmer et al., *sCD25 as an independent adverse prognostic factor in adult patients with HLH: results of a multicenter retrospective study* © 2023 by Elsevier [2], permission of the publisher as author of the article not required).

Agent	Total N (%)	Malignancy	Infection	Autoimmune	Idiopathic
Glucocorticoids	61 (98)	22 (100)	21 (95)	10 (100)	8 (100)
Dexamethasone	45 (74)	18 (82)	17 (81)	3 (30)	7 (88)
Prednisolone*	16 (26)	4 (18)	4 (19)	7 (70)	1 (13)
Etoposide	27 (44)	11 (50)	9 (41)	2 (20)	5 (63)
Cyclosporine A	14 (23)	4 (18)	5 (23)	2 (20)	3 (38)
Rituximab	16 (26)	6 (27)	10 (45)	0 (0)	0 (0)
i.v. immunoglobulins	19 (31)	5 (23)	10 (45)	0 (0)	4 (50)
Cyclophosphamide	13 (21)	8 (36)	2 (9)	3 (30)	0 (0)
Anakinra	9 (15)	1 (5)	3 (14)	5 (50)	0 (0)
Tocilizumab	2 (3)	1 (5)	0 (0)	1 (10)	0 (0)
Alemtuzumab	1 (2)	0 (0)	1 (5)	0 (0)	0 (0)
Ruxolitinib	1 (2)	0 (0)	1 (5)	0 (0)	0 (0)
Cytokine adsorption	9 (15)	4 (18)	4 (18)	0 (0)	1 (13)
Total	62	22	22	10	8

* Only patients who did not receive additional dexamethasone

i.v., intravenous

3.5.1 Glucocorticoids

Sixty-one patients (61/62, 98%) received HLH specific immunosuppressive treatment with glucocorticoids: dexamethasone was administered to 45 patients (45/61, 74%), whereas 16 individuals (16/61, 26%) received (methyl-)prednisolone. [2]

Of note, 23 patients (23/62, 37%) were treated with empirical glucocorticoid therapy before HLH was suspected and the treatment scheme adopted accordingly.

3.5.2 HLH-94/2004 protocol

Etoposide was administered to 27 (27/62, 44%) and cyclosporine A to 14 (14/62, 23%) patients, respectively. Eleven patients (11/62, 18%) received all three main agents of the HLH-94/2004 protocol (dexamethasone, etoposide and ciclosporin). Two patients (2/62, 3%) received an intrathecal therapy containing dexamethasone, cytarabine, and methotrexate.

3.5.3 Further immunosuppressive agents and chemotherapies

Sixteen patients (16/62, 26%) received rituximab. The main reason for its administration was the association with EBV: ten patients (10/16, 63%) had an active EBV infection, five (5/16, 31%) EBV-associated malignancies, and one patient (1/16, 6%) a B-cell lymphoma. IVIGs were given to 18 patients (18/62, 29%). The cytostatic agent cyclophosphamide was administered to 13 (13/62, 21%), the IL-1 receptor antagonist anakinra to nine (9/62, 15%), and the monoclonal IL-6 receptor antibody tocilizumab to two patients (2/62, 3%), respectively. The Janus kinase inhibitor ruxolitinib and the monoclonal CD52 antibody alemtuzumab were used as adjunctive treatment in one patient (1/62, 2%). [2]

Six patients (6/62, 10%) received some of the aforementioned agents alongside standardized chemotherapy protocols: R-CHOP (**R**ituximab – **C**yclophosphamide, **H**ydroxydaunorubicin (i.e. doxorubicin), **O**ncovin (i.e. vincristine), **P**rednisolone) was administered to three patients (etiologies: EBV-positive Hodgkin lymphoma, B-cell lymphoma, polymorph EBV-associated lymphoproliferation, respectively), and each one patient received a treatment according to the R-CHOEP (R-CHOP + **E**toposide; etiology: EBV-positive NK-cell leukemia), CHOEP-14 (etiology: T-cell lymphoma), and BEACOPP (**B**leomycin, **E**toposide, **A**driamycin (i.e. doxorubicin), **C**yclophosphamide, **O**ncovin (i.e. Vincristine), **P**rocarbazine, **P**rednisolone; etiology: EBV-positive Hodgkin lymphoma) scheme, respectively. [2]

3.5.4 Stem cell transplantation (SCT)

A 19-year-old male patient with a nonsense mutation in the *XIAP*-gene (see chapter 3.2.7 *Genetics*) developed an HLH recurrence and therefore received alloSCT four months after the first EBV-triggered HLH episode. The alloSCT lead to a clinical remission which was maintained until the end of the study period (95 days after the SCT). [2]

3.5.5 Cytokine adsorption

A cytokine adsorber was installed in nine patients (9/62, 15%) who were in need of an external blood circulation during their HLH episode [2].

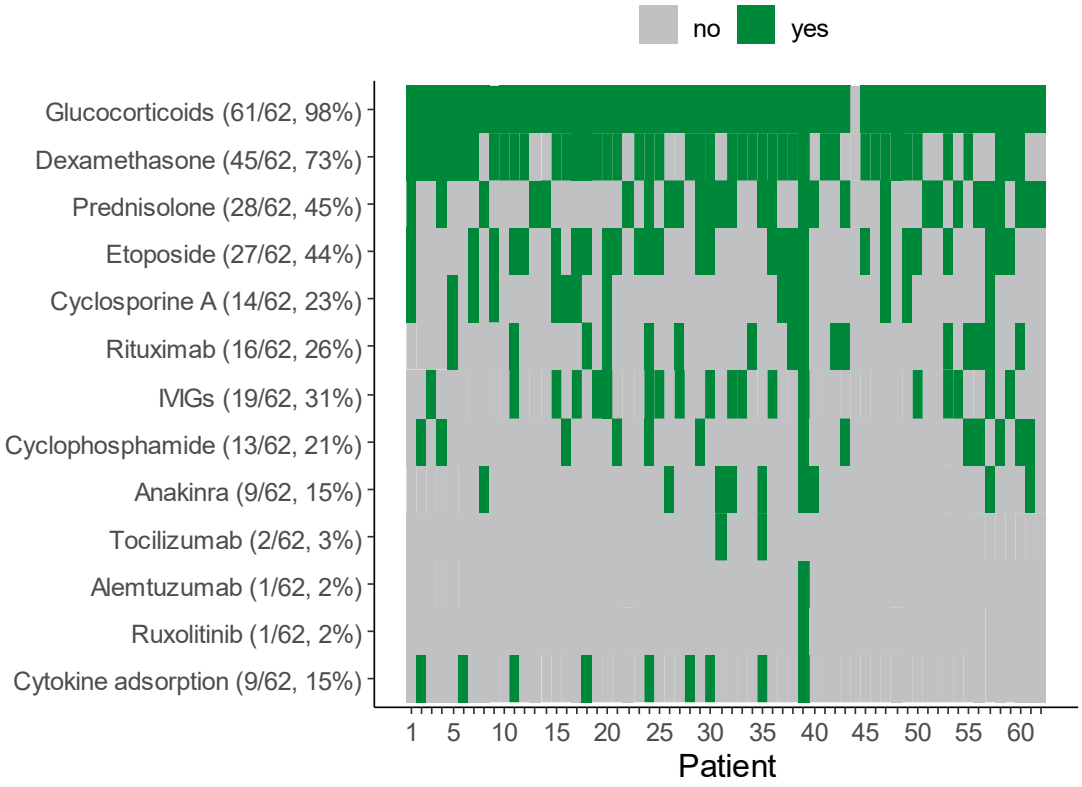

Figure 19 Heat map depicting agents used for HLH treatment per patient. Patient 44 suffered from CMV associated HLH and was successfully treated with intravenous ganciclovir. *IVIGs*, intravenous immunoglobulins. (Wimmer et al., *sCD25 as an independent adverse prognostic factor in adult patients with HLH: results of a multicenter retrospective study* (supplement) © 2023 by Elsevier [2], permission of the publisher as author of the article not required).

3.6 Transfused blood products

Fifty-seven (57/62, 92%) patients received a transfusion of blood products during their first HLH associated hospital stay. The most common ones were concentrates of erythrocytes (48/62, 77%) and thrombocytes (37/62, 60%). An overview of the kind and amount of transfused blood products is shown in Table 7.

Table 7 Overview of transfused blood products

Type	N	(%)	Median	Range
Erythrocyte concentrates [n]	48	(77)	6	1-117
Thrombocyte concentrates [n]	37	(60)	11	1-70
Plasma substitutes [n]	14	(23)	20.5	2-213
Albumin [g]	17	(27)	120	40-2,410
Fibrinogen [g]	22	(35)	14.5	2-138
Prothrombin complex concentrate [IE]	19	(31)	4,800	1,000-30,000
Antithrombin [IE]	12	(19)	2,000	500-27,000
Factor XIII [IE]	9	(15)	1,250	1,250-5,500

3.7 Outcome

Median survival of the population was 288 days (0.79 years), and one year survival probability was 49.5%. A Kaplan-Meier curve depicting the survival probability of the study population is shown in Figure 20. Overall mortality surpassed 50%: 32 patients (32/62, 52%) had a documented death of any cause until the end of the study period.

The most common cause of death in the deceased patients was multi-organ failure, present in 19 cases (19/32, 59%). Leading renal failure and cardiovascular failure due to lung edema alongside pneumonia was described in one patient each (1/32, 3%, 1/32, 3%). The remaining patients died either because of infections (6/32, 19%), HLH (3/32, 9%), NK/T-cell lymphoma (1/32, 3%), and bleeding (1/32, 3%).

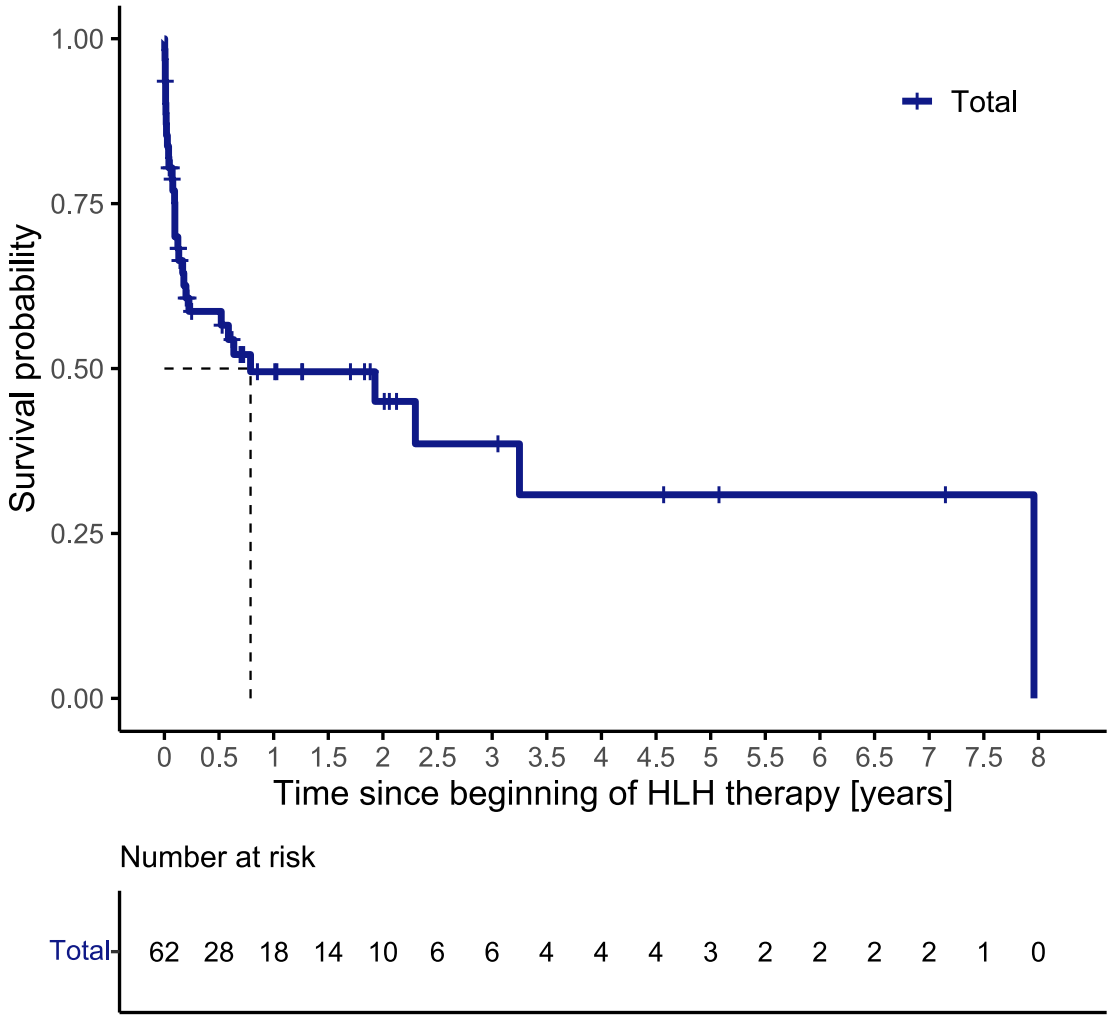

Figure 20 Kaplan-Meier curve of the total study population. Median survival of the population was 288 days (0.79 years), and one year survival probability was 49.5%.

3.8 Prognostic Factors

Univariate and multivariate Cox-regression models were performed in order to detect possible prognostic factors addressing overall survival. In the univariate analysis the following parameters around treatment initiation were significantly ($p < 0.1$) associated with worse overall survival: older age, malignant triggers, elevated serum levels of AST, creatinine, INR, LDH, sCD25, and lowered platelet count and albumin level. In the multivariate analysis, only elevated sCD25 values remained statistically significant ($p = 0.005$) (Table 8). [2]

In order to compare the survival probability of the four etiologic subgroups, Kaplan-Meier curves were drawn (Figure 21). The accompanying log-rank test showed a significant difference (p = 0.004) between the four etiologic subgroups.

The significantly worse prognosis of patients with malignancy triggered HLH is shown in a Kaplan-Meier curve in which M-HLH patients were opposed to the other three etiologic groups (p = 0.0007, log-rank test) (Figure 22). [2]

Table 8 Results of the Cox-Regression models (Wimmer et al., *sCD25 as an independent adverse prognostic factor in adult patients with HLH: results of a multicenter retrospective study* © 2023 by Elsevier [2], permission of the publisher as author of the article not required).

	Univariate analysis			Multivariate analysis		
Parameter	Hazard Ratio	95% CI	p	Hazard Ratio	95% CI	p
Age	1.02	1.00 – 1.04	0.06	1.03	0.98 – 1.08	0.25
Male Sex	1.89	0.81 – 4.40	0.14			
Malignancy	3.40	1.61 – 7.19	0.001	1.92	0.43 – 8.64	0.39
Albumin	0.54	0.28 – 1.04	0.07	0.56	0.17 – 1.88	0.35
AST*	1.02	1.00 – 1.05	0.07	1.00	0.95 – 1.05	0.96
Creatinine	1.32	1.04 – 1.68	0.02	1.16	0.71 – 1.92	0.55
Ferritin†	1.01	0.91 – 1.11	0.93			
Fibrinogen	1.00	1.00 – 1.00	0.24			
Hemoglobin	0.93	0.77 – 1.13	0.48			
INR	3.24	1.50 – 7.00	0.003	1.07	0.28 – 4.06	0.92
LDH†	1.15	1.02 – 1.29	0.02	1.27	0.95 – 1.70	0.11
Leukocytes	1.01	0.97 – 1.05	0.65			
Neutrophils	1.02	0.97 – 1.07	0.55			
Platelets	0.99	0.98 – 1.00	0.005	1.00	0.99 – 1.01	0.76
sCD25‡	2.40	1.67 – 3.44	<0.0001	2.22	1.28 – 3.87	0.005
Triglycerides	1.00	1.00 – 1.00	0.78			

* Absolute laboratory values divided by 100 before entering to the Cox regression model
† Absolute laboratory values divided by 1,000 before entering to the Cox regression model
‡ Absolute laboratory values divided by 10,000 before entering to the Cox regression model
AST, Aspartate transaminase; *CI*, confidence interval; *INR*, international normalized ratio; *LDH*, lactate dehydrogenase; *sCD25*, alpha chain of the soluble interleukin-2 receptor.

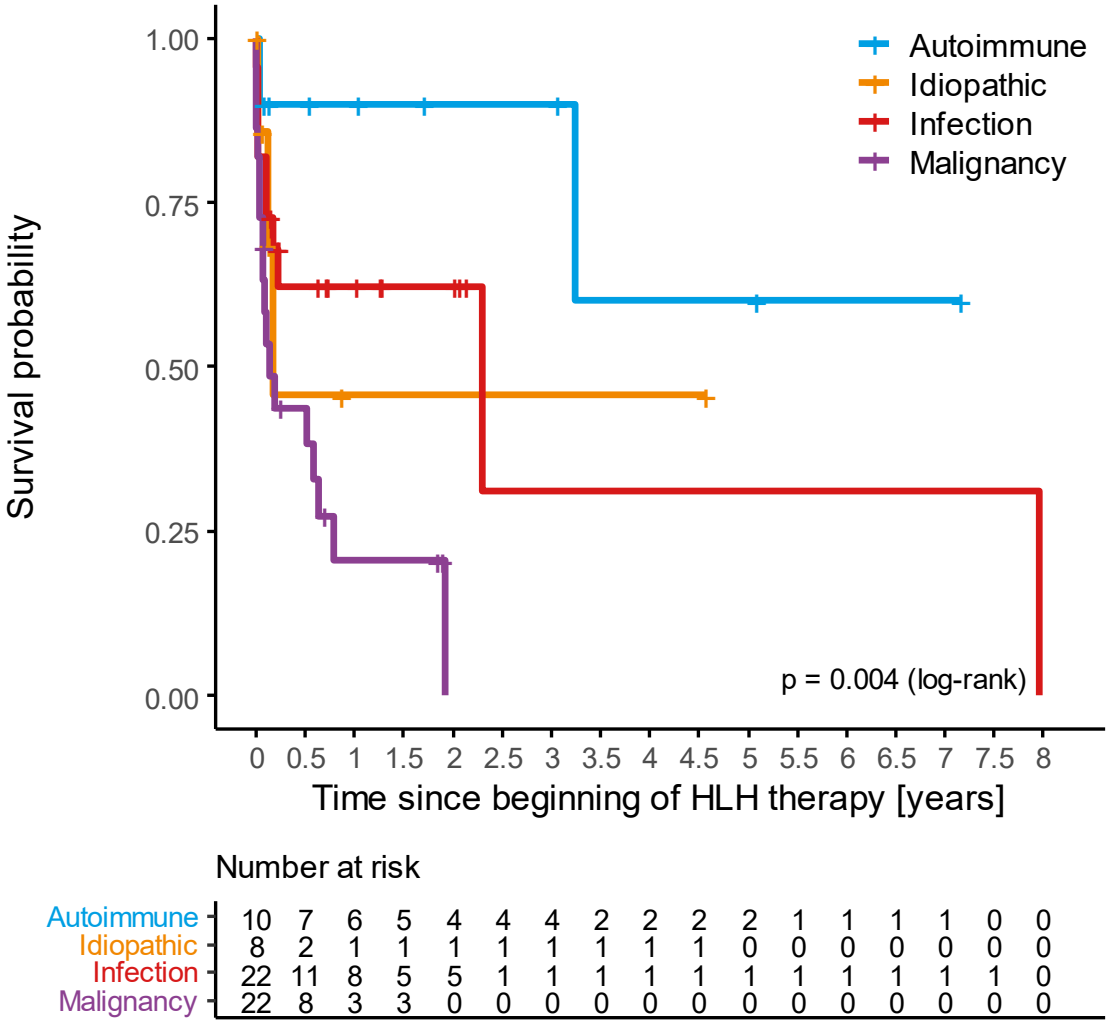

Figure 21 Kaplan-Meier curves comparing the four etiologic subgroups (Wimmer et al., *sCD25 as an independent adverse prognostic factor in adult patients with HLH: results of a multicenter retrospective study* © 2023 by Elsevier [2], permission of the publisher as author of the article not required).

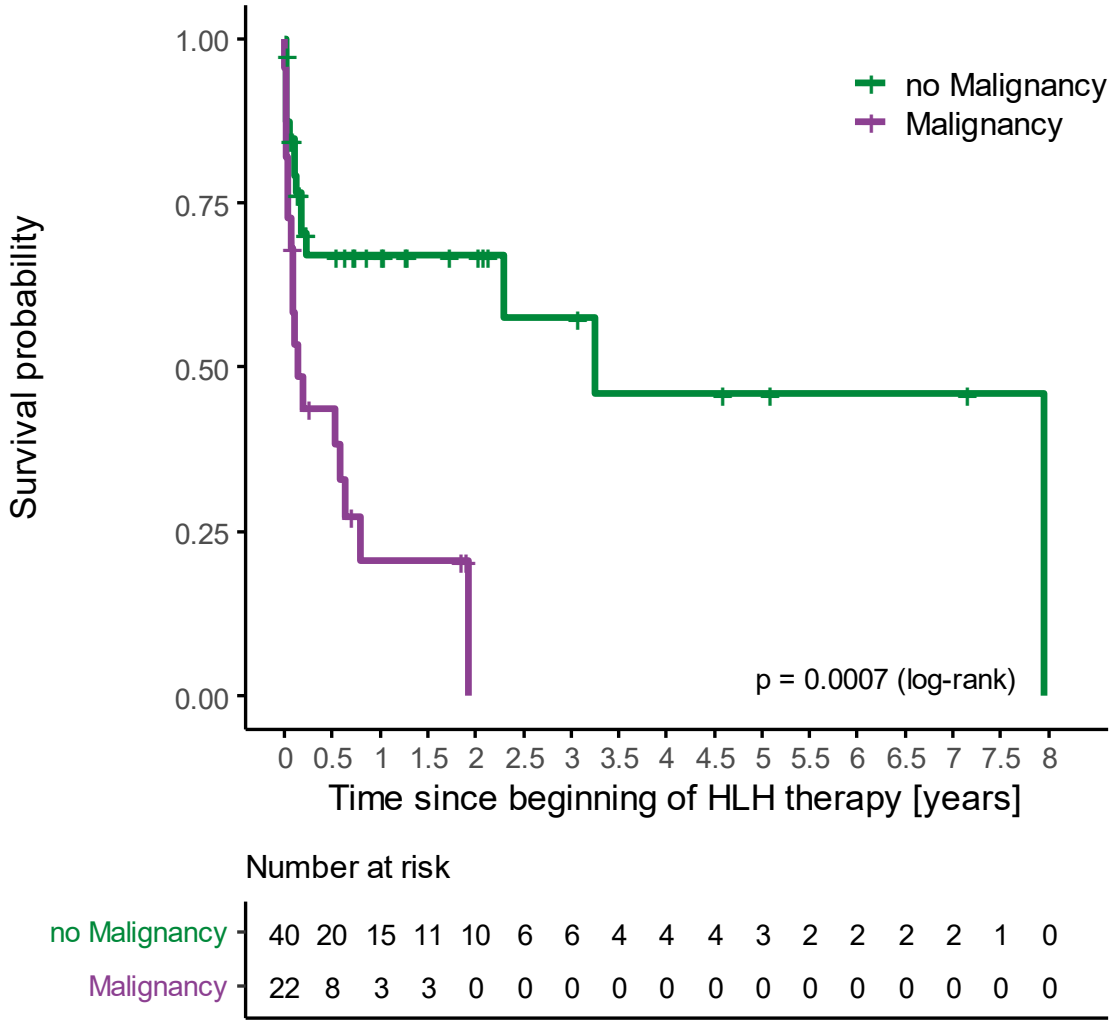

Figure 22 Kaplan-Meier curve comparing M-HLH patients with other etiologies

3.8.1 Exemplary comparison between the course of ferritin in two individual patients with different outcome

In order to showcase the importance of also regarding the relative course of selected possible prognostic factors during the HLH episode (see also chapter 4.6.2), an exemplary comparison between the course of ferritin of two patients with different outcome is depicted in Figure 23. One can see that the surviving patient, a 35-year-old female with idiopathic HLH, presented with a higher ferritin level at treatment initiation, which decreased after administration of an HLH-directed therapy. In contrast, the comparably low ferritin level around treatment initiation of the deceased patient, a 24-year-old male with EBV-triggered HLH, increased over the course of the HLH episode.

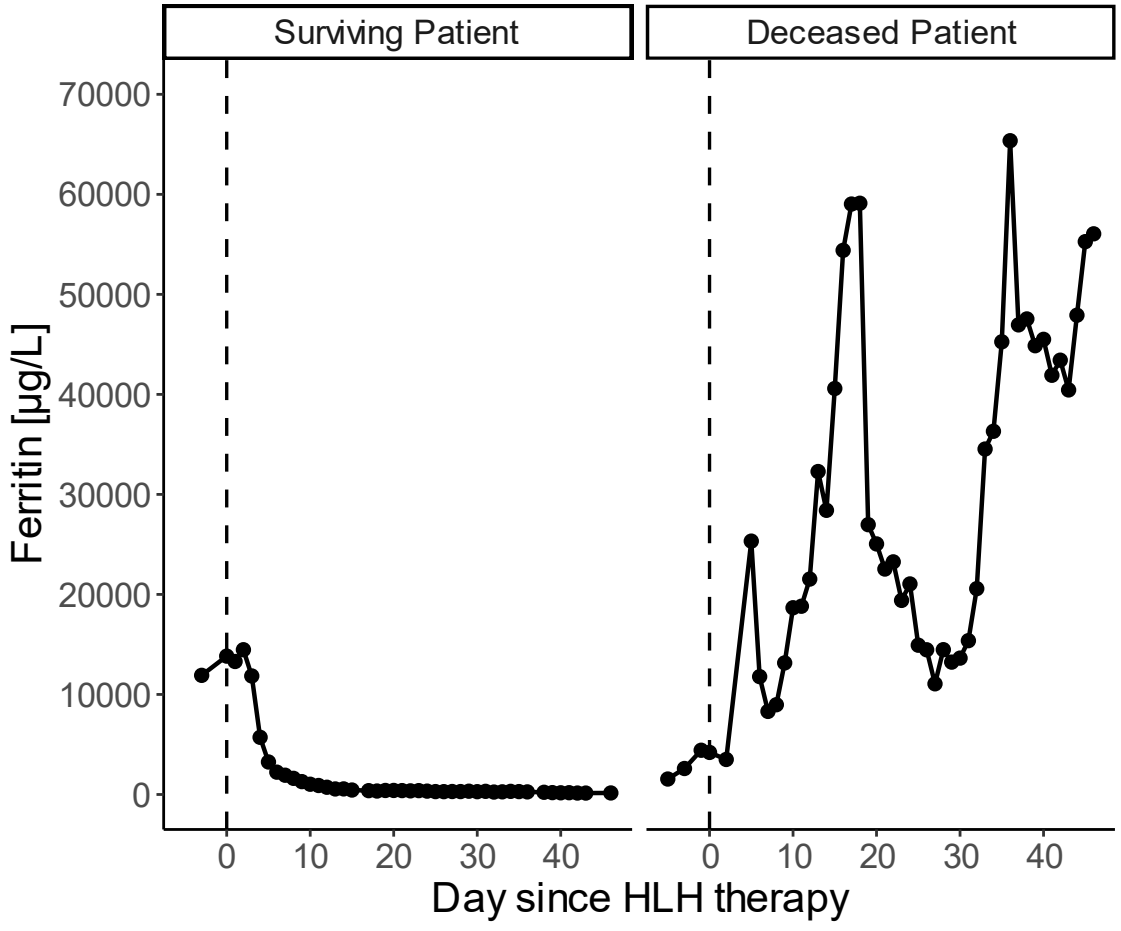

Figure 23 Comparison of the ferritin course of two patients with diverse outcome.

4. Discussion

4.1 Epidemiologic data

The epidemiologic data of the study population is generally in accordance with previous case series on adult HLH: the median age of 53.5 years, a male to female ratio > 1 (1.82:1), as well as a predominance of infectious and malignant triggers is fitting in well with previously published data [8, 68, 81, 82, 101, 115]. Likewise, the significant difference in overall survival according to the most likely HLH trigger is conform with previous studies. Of special note once again is the poor prognosis of patients with malignancy associated HLH (see Figure 22): the one-year survival probability was 21% and overall mortality in this subgroup reached 77%. Another interesting finding in the malignant subgroup was the concomitance of EBV-associated neoplasms: eight patients (8/22, 36%), among them all four cases of primarily Hodgkin lymphoma triggered HLH, had an EBV-associated malignancy. Associations of EBV with HLH triggered by Hodgkin lymphoma have previously been described in up to 90% of cases [36, 69]. This once again underlines the importance of this herpesvirus in HLH pathophysiology.

The predominant trigger in the autoimmune subgroup was AOSD (5/10, 50%). In contrast to several previous studies, where SLE was the predominant trigger of MAS-HLH [64, 80, 115], no patient with SLE associated HLH had been included in this study. The exact reasons for this remain unclear, but can likely be traced back to the small sample size and the search algorithm, which was primarily performed within hematologic/oncologic departments.

4.2 Diagnostic criteria

4.2.1 Histopathologic proof of hemophagocytosis

Rate of histopathologic hemophagocytosis in the bone marrow examinations (histology and cytology) of the present population was 58%. A similar proportion has been published by the German HLH register, where features of hemophagocytosis were present in 63% of the population [81]. In three further large retrospective studies, rates of hemophagocytosis ranged from approximately 40 to 70% [20, 68, 115]. With this data in mind, it is important to note that the singular presence or absence of hemophagocytosis does neither rule out, nor confirm HLH diagnosis, even though being the eponymous symptom of the

syndrome [145, 158-160]. Especially at onset of disease, histopathologic proof of hemophagocytosis can be lacking [100]. In addition, hemophagocytosis has been described in severely ill patients without accompanying HLH in up to two-thirds of cases [161, 162].

Thus, despite the fact that hemophagocytosis was a mandatory feature for the diagnosis of HLH prior to the updated HLH-2004 criteria [23], given the often-missing proof of it, combined with the limited sensitivity and specificity, its overall diagnostic importance has decreased within the last years. Consequently, Jordan et al. referred to it as "one of the less important diagnostic criteria" [5] and Cetica et al. proposed its overall removal from the diagnostic criteria [20].

4.2.2 Clinical HLH triad

The so-called clinical HLH triad consisting of fever, splenomegaly, and a bi-/pancytopenia [61], was present in 55 patients (55/62, 89%): similar to previous publications, almost all patients suffered from fever (60/62, 97%) [8, 81]. Of note, the remaining two patients also had an elevated body temperature of 37.8 °C each. Therefore, the presence of an HLH can be regarded as unlikely in patients without accompanying fever/elevated body temperature.

Because of the differences considering cytopenia's cutoffs and incorporated type of white blood cells between the HLH-2004 criteria and the HScore, there was a gap of 20% with regard to the presence of a bicytopenia between these two diagnostic algorithms in the population (69% vs. 89%). Since the HScore was developed based on an adult population and the cutoff values were determined statistically, it probably represents the better fit for HLH patients of different etiologies ≥ 18 years as is the case in the present study. Furthermore, the incorporation of leukocytes instead of neutrophil granulocytes is more practicable in the clinical setting, since a standard blood count is sufficient for its measurement.

4.2.3 Ferritin cutoff

There has been criticism concerning HLH-2004's ferritin cutoff at 500 µg/L for being too low and therefore not specific enough in children and adults alike [163]. One retrospective pediatric study described a 90% sensitivity and 96% specificity for an adapted ferritin

cutoff at 10,000 µg/L [164]. However, in adults, even values above 50,000 µg/L were lacking specificity [165, 166].

Since all 62 patients showed elevated values > 500 µg/L, it represents the most frequently fulfilled diagnostic criterion. In addition, as the majority of the population also surpassed the two higher cutoffs of the HScore at 2,000 and 6,000 µg/L, our data underline that a higher cutoff in the adult population, such as the recently suggested 2,000 µg/L [167], seems reasonable. Furthermore, despite its lack of specificity, the absence of moderately elevated ferritin values > 500 µg/L should question the presence of HLH in adults.

4.3 Genetic findings

Mutations in HLH related genes were found in 8% (5/62) of the study population, or in 56% (5/9) who underwent adequate testing. Considering that the population solely consists of adults ≥ 18 years, this finding marks a considerable rate. However, by analogy with the inversely correlation between occurrence of primary HLH and age at onset of disease [23], the mutations in our population were generally associated with less drastic functional deficits.

4.3.1 *PRF1* Ala91Val variant

Of the two patients, who were positively tested for biallelic mutations in the *PRF1* gene, a 51-year-old male presented a homozygous Ala91Val variant. It has been discussed whether this variant represents a simple polymorphism or is indeed associated with a higher susceptibility towards HLH. Supporting facts for the first assumption have been proposed by zur Stadt et al., who detected a heterozygous Ala91Val variant in 15 of 86 (17%) healthy Caucasians [168]. They concluded that the prevalence of HLH had to be far higher in Germany, if this variant was associated with HLH manifestation, even when assuming an allele frequency of 9% [168]. Subsequent studies, however, showed that the frequency of alleles carrying the Ala91Val variant actually was significantly higher in populations with HLH association than in control groups [169, 170]. Moreover, it has been discovered that the Ala91Val variant impairs perforin function and therefore lymphocyte toxicity [171, 172]. This led to the assumption that the susceptibility of developing HLH, especially in cases of homozygous or combined heterozygous states of Ala91Val, is indeed increased [20, 169]. Since the amino acid substitution from alanine to valine is resulting in a remaining rest activity of the perforin protein, this variant is

generally associated with late onset HLH [28]. Likewise, the first publication of adult onset FHL reported two siblings who developed HLH in their twenties and carried a combined heterozygous Ala91Val and Trp374Stop mutation in the *PRF1* gene [173]. The authors suggested that the late HLH onset could be associated with the Ala91Val variant and concluded that a milder course of HLH disease activity is likely to occur in patients with homozygous Ala91Val alleles. This being a finding that has not yet been made at the time of publication [173]. Eventually, the first case description of HLH associated with homozygous Ala91Val alleles had been published four years later, and described a 49-year-old male who developed HLH on the ground of a chronic tuberculosis infection [174]. When comparing this patient to the one in the present population, some similarities and differences become apparent: besides the male sex, age of initial HLH onset was strikingly similar with 49 and 51-years, respectively. The older age at onset underlines that a perforin dependent cytotoxic rest-activity had to be present. Likewise, the results of immunologic tests in our patient's NK cells showed a reduced but detectable perforin expression and a normal NK cell degranulation after stimulation with IL-2. Differences between these two patients become evident when regarding etiology and outcome: whereas no specific HLH trigger could be detected in the patient of the present cohort, the one described in the literature was diagnosed with a chronic tuberculosis infection and was under glucocorticoid therapy. Concerning outcome, the patient in the present population did not need alloSCT, even though a recurrent HLH episode occurred 2.5 years after the initial one, since he showed a repeatedly good therapy response to glucocorticoids and etoposide, and had a stable clinical condition under continuation therapy with cyclosporine. In contrast, the patient in the literature died because of his HLH episode. Therefore, only the patient of the present population is fulfilling the prediction of a milder disease course, which had been proposed by Clementi et al. [173]. The overall rareness of homozygous Ala91Val alleles has been confirmed in a recent meta-analysis with 391 HLH cases and 975 controls, where only two individuals in the HLH and three in the control group presented with a homozygous Ala91Val variant [170].

4.3.2 Additive heterozygous mutations

By analogy with the aforementioned Ala91Val variant of the *PRF1* gene, there is evidence that heterozygous mutations in FHL related genes are also associated with late onset FHL. Likewise, in a large study on genetics in adult HLH, 25 of 175 (14%) patients ≥ 18 years had either missense or splice-site change mutations in the *PRF1* (FHL2),

UNC13D (FHL3), and *STXBP2* (FHL5) genes [7]. The role of additive heterozygous mutations in HLH was further consolidated in a murine model, where animals with concomitant heterozygous mutations in different FHL related genes developed HLH-like symptoms after being infected with lymphocytic choriomeningitis virus [175]. Likewise, one patient in the present study was diagnosed with two heterozygous variants of unclear significance in each the *UNC13D* (FHL3) and *STXBP2* (FHL5) gene, after having developed an HLH episode triggered by a primary EBV-infection.

Thus, even though not being singularly responsible for developing HLH, an increased susceptibility due to the *PRF1* Ala91Val variant and heterozygous mutations in FHL related genes, seems reasonable (see Supplemental Figure 1).

In summary, genetic testing in adult HLH patients, in particular under presence of certain red flags such as young age, male sex, EBV-association, recurrent HLH episodes, and family history of HLH, should be considered [61]. Especially the use of WES could prove very promising in detecting new genetic anomalies which are associated with the occurrence of (late-onset) HLH [176].

4.4 Diagnostic value of metabolic imaging

A murine model investigating the ^{18}F-FDG uptake in tumoral tissues showed that the highest uptake took place in activated macrophages rather than in the tumor cells themselves [177]. PET-CT also proved to be of value in detecting malignancies, especially lymphoma [102, 154] and underlying causes of patients suffering from fever of unknown origin (FUO) [178]. Therefore, metabolic imaging technics such as PET-CT in theory appear to be useful in detecting the extent of HLH disease, as well as helpful to identify an underlying trigger. The diagnostic value of metabolic imaging in HLH patients was evaluated by reviewing the findings of all 26 patients, who received a metabolic imaging procedure during their HLH episode (25 PET-CT, 1 full-body scintigraphy).

The most frequent findings, which likely represent HLH/macrophage activity, consisted of increased ^{18}F-FDG uptake in lymph nodes, bone marrow, and spleen. Previous studies reported similar findings, especially a diffuse uptake of ^{18}F-FDG in the bone marrow [102, 154, 179-181]. Additionally, a significant worse outcome has been described in two studies of patients with an increased SUVmax in the bone marrow [179, 182]. Whether these findings represent genuine HLH activity, or can rather be traced back to underlying malignancies or reactive bone marrow hematopoiesis [102], still remains unclear. In the

present study population, metabolic imaging did not allow for a single initial diagnosis of an underlying malignancy nor the detection of an infectious HLH trigger, including the results of subsequent punctures in suspected tissues. In the literature, PET-CT was useful in detecting the underlying HLH trigger in 65% (28/43; 25/28 lymphoma) [154] and 23% (6/26; all 6 lymphoma) [101] of cases. Sensitivity of PET-CT in detecting an underlying malignancy in HLH was 83% (5/6), with a failure to detect one case of T-cell lymphoma [182]. However, in another study on adult HLH, positive PET-CT findings being defined as an increased ^{18}F-FDG uptake, were only detected in a minority of 49% (22/45): in patients with lymphoma associated HLH, the rate of positive findings was 46% (13/28), and 50% (5/10) in patients with an infectious triggered HLH, respectively [180]. A recent meta-analysis, including a total of 300 patients of ten retrospective studies, showed that HLH patients with a malignant trigger presented with statistically higher SUVmax in the spleen, bone marrow, and lymph nodes compared to patients with benign triggers [103]. A significant higher SUVmax in patients with lymphoma associated HLH had also been reported previously [102, 183]. Thus, PET-CT can be valuable for differentiating between HLH triggered by malignancies and other etiologies. However, for the final differentiation and confirmation of the underlying trigger, a biopsy of the suspected tissue remains mandatory [180]. PET-CT can also be valuable for the detection of a suitable biopsy site [103].

In summary, metabolic imaging technics such as PET-CT can be considered in patients with an unknown HLH trigger, especially when the suspicion of an underlying and yet undetected malignancy arises [61].

Of note, since elevated CRP values have been found to correlate with a higher SUV in the bone marrow and positive findings in HLH patients [154], this routinely measured laboratory parameter might help in the decision making regarding the use of metabolic imaging.

The lack of diagnostic findings in the present study population may be traced back to the previous (glucocorticoid) treatment and the absence of a stringent diagnostic work-up that comes along with the retrospective study design.

4.5 Effect of therapy

With regard to the retrospective study design which did not allow to randomly address patients to different treatment groups, the comparison of the performed therapies and their effect on outcome must be considered with caution. It can be assumed that more severe cases received more intensive treatments, such as an etoposide containing regime. Therefore, a clear bias towards a worse outcome is inevitable. Likewise, patients who received etoposide had a significant worse outcome in comparison to patients who did not (Supplemental Figure 2). With regard to the known benefits of etoposide in pediatric [12, 93] as well as adult populations [138, 184], this finding can likely be traced back to a treatment bias following the need of a therapy escalation because of severity of disease or lacking response to less toxic therapies. Finally, in contrast to previous case descriptions [126-128], only one of the nine patients who were treated with a cytokine adsorber survived within the study period. Since such an adsorber can only be applied when an external blood circuit is established, as is for example the case during renal replacement therapy, there was probably a bias towards more severe ill patients with renal-/multi-organ failure. Thus, it was not reasonably possible to give therapy recommendations based on our data, as we had initially intended.

Considering the role of novel therapeutic agents, it should be noted that only one patient received adjunctive treatment with the JAK 1 + 2 inhibitor ruxolitinib. Ruxolitinib showed promising results in pediatric and adult HLH patients [185, 186] and as an additional agent used for salvage therapy [119]. Another promising therapeutic option in the future, especially with regard to the pivotal role that IFNγ plays in HLH pathophysiology (see chapter 1.3), could be the monoclonal IFNγ antibody emapalumab. Accordingly, emapalumab has shown benefits in treating children < 14 years with genetic HLH [122] and has been approved for this entity in children by the U.S. Food and Drug Administration in November 2018 [187]. An increasing use in children and adults alike, also in other countries, seems conceivable and may therefore, together with a growing administration of agents such as ruxolitinib, improve the overall outcome of HLH patients in the future.

4.6 Diagnostic and prognostic value of sCD25 and ferritin

Since sCD25 and ferritin are marking two comparably specific laboratory parameters for HLH, both of which also proved to be of special diagnostic value [188, 189], their diagnostic and prognostic characteristics shall be discussed as follows.

4.6.1 Role of sCD25 in HLH

4.6.1.1 Diagnostic value of sCD25

Data addressing sCD25's value in diagnosing HLH have been increasing over the last years: Hayden et al. stated in their scoping review on adult HLH in 2016 that sCD25 is a comparably low-cost laboratory parameter, whose value as a disease marker should be addressed in further studies [23]. Consequently, Hayden et al. showed in a subsequent study that the sensitivity of sCD25 in diagnosing HLH reached an area under the curve (AUC) of 0.90, which was higher than the AUC of ferritin alone (0.78), as well as the AUC of a combination of sCD25 and ferritin (0.88) [107]. Likewise, Damoiseaux stated in his publication about the sCD25 pathway that sCD25 measurement is very useful in diagnosing HLH, even though he did not address any prognostic significance to it [56].

4.6.1.2 Prognostic value of sCD25

In the present study, sCD25 showed the lowest p-value ($p < 0.0001$) in the univariate Cox-regression model and remained the only significant prognostic parameter for overall survival in the multivariate analysis ($p = 0.005$). This finding is of special interest since data describing the prognostic value of sCD25 in adult HLH patients have been rare. The main reason for this is the limited laboratory availability in previous studies [8, 23, 64, 67, 71, 83, 86, 87, 141, 142, 144, 152, 190]. [2]

In the pediatric population, however, Imashuku et al. proposed a prognostic value of sCD25 already in the 1990s [147, 148]. Regarding the literature on adult HLH, one study with 35 patients described significantly higher sCD25 values in patients with EBV and malignancy associated HLH, and an association of elevated sCD25 values with worse overall survival [149]. In a further retrospective study describing 31 adult HLH patients, where sCD25 was measured in 19 individuals, a significantly higher mortality had been described in patients with elevated maximum sCD25 values during their HLH episode. [150]. [2]

4.6.1.3 sCD25 as a marker of HLH disease activity

Since previous studies showed that HLH is accompanied and maintained by an excessive activation of T-cells [48, 191], which is leading to the shedding of sCD25 [56], it seems reasonable that sCD25 could not only serve as a marker for T-cell activation, but also for HLH activity. This judgement is shared by Jordan et al., who addressed sCD25 in HLH

as “one of the most useful inflammatory markers, as it correlates with current disease activity more consistently than ferritin or other disease indices” [5]. With regard to the findings in the present study, the determination of sCD25 should be considered without delay whenever suspicion of HLH arises. [2]

4.6.1.4 Therapeutic consequences

Given the dismal prognosis of patients with highly elevated sCD25 levels in the present population, the question arises whether the early application of T-cell targeting substances may be valuable in selected cases. Besides etoposide, which showed a primarily T-cell depleting effect in the murine model [111] and improved the short time survival in adults when applicated within the first two weeks after HLH diagnosis [138], second line substances such as the monoclonal anti-CD52 antibody alemtuzumab [123] or anti-thymocyte globulin (ATG) could be also considered [191, 192]. [2] Whether sCD25, which is thought to have a serum-level dependent function on immune-regulation [56], is also playing a pathophysiological role in the development of HLH is not exactly known. Therefore, it also remains unclear whether monoclonal sCD25 antibodies may be helpful as additional agents in the treatment of selected patients outside single case descriptions [124, 193]. Further studies on this topic, preferably prospective ones, are needed to address these questions.

With regard to sCD25 representing the extent of T-cell activation, and ferritin macrophage activity, the elevation of one or another parameter could also help in addressing tailored therapies [190].

4.6.2 Prognostic value of ferritin

In the univariate Cox-regression model there was no significant association between the respective ferritin levels around the beginning of HLH-specific therapy and overall survival ($p = 0.93$). Nevertheless, strongly elevated ferritin values often raise first suspicion towards the possibility of an active HLH in the clinical setting [194]. Given its characteristic as a marker for macrophage activation in HLH [66, 195], it is tempting to also address HLH disease activity and a prognostic value to it. [2] Moreover, previous studies have shown a significant correlation between elevated ferritin levels and outcome (see chapter 1.7.2). However, the results of several studies with comparable large populations are congruent with ours [84, 138]. Furthermore, in two studies with each over 100 patients, ferritin showed a prognostic significance only in the univariate but not in the

multivariate analysis [81, 141]. Even with regard to these diverging results, given its widespread and low-cost laboratory availability [196], ferritin nonetheless appears to be the preferred marker for observing therapy response in HLH [195, 197]. In line with that, two studies showed that the ratio of ferritin decline during the HLH disease course may have a prognostic value [155, 156]. Likewise, one recent study consisting of 124 adult HLH patients of various etiologies described that the course of the ferritin level was the paramount risk factor in the multivariate analysis concerning 30- and 100-day short time survival. However, neither initial nor maximum ferritin levels correlated with 30-day mortality in patients with malignancy associated HLH (n = 43) [115]. [2]

Similar results were obtained in a further study with 102 adult HLH patients of different triggers, where no significant difference in the ferritin values at treatment initiation were found between the remission and non-remission group [116]. With regard to long-term survival, there was no significant difference concerning ferritin values measured within the first two weeks of treatment. However, ferritin values at weeks three and four were significantly associated with overall survival [116]. Thus, the serial measurements of ferritin in order to assess therapy response and HLH disease activity seems practical, but the prognostic significance of single absolute values, in particular around diagnosis/treatment initiation, should not be overestimated. [2]

Even more so, since serum ferritin levels are susceptible to external influences, for example through the transfusion of blood products, which are often applied in HLH patients [190] (see chapter 3.6).

The exemplary ferritin course of two patients during their HLH episode, which underline aforementioned theses on the prognostic value of ferritin, is shown in Figure 23.

4.6.3 Diagnostic and prognostic value of sCD25 and ferritin combined

The combined consideration of sCD25 and ferritin is of special interest in patients with lymphoma associated HLH: these showed to have a significantly higher ratio of sCD25 towards ferritin when compared to HLH patients of other etiologies [198, 199]. Recently, Zoref-Lorenz et al. retrospectively analyzed the ferritin and sCD25 levels of 225 patients of an international study population with hematologic malignancies; among them were 112 individuals with accompanying HLH [188]. Through the combination of sCD25 values > 3,900 U/mL and ferritin > 1,000 µg/L, they introduced the *optimized HLH inflammatory* (OHI) index. The OHI index proved to be useful concerning diagnosis and

prognosis in the HLH subgroup [188]. Furthermore, the OHI index also showed a prognostic significance in patients with hematologic malignancies without accompanying HLH [188]. Therefore, the routine measurement of sCD25 and ferritin in patients with hematologic malignancies, even without the suspicion of an accompanying HLH, should be debated and addressed in further studies. [2]

Regarding the present study population, the OHI index did not show significant results in the univariate Cox-regression model ($p = 0.15$). This can be traced back to the fact that a majority of patients with sufficient data met the requirements of the index (34/42, 81%). All 42 patients presented ferritin levels > 1,000 µg/L. Therefore, the OHI index allowed the dichotomization of the study population solely based on the sCD25 threshold at 3,900 U/mL. Since this threshold is considerably lower than the median sCD25 level of the study population at 7,501 U/mL, the lacking significance in the Cox-regression model is to be expected. Thus, the consideration of continuous sCD25 levels as presented in the uni- and multivariate Cox-regression analyses mark a more simple and aptly prognostic model in the present study population.

4.7 Limitations

The major limitation of this study lies within its retrospective approach which lead to a limited availability of data. Likewise, only 39 patients had sufficient laboratory data to be incorporated in the multivariate Cox-regression model (etiologies: autoimmune n = 6, idiopathic n = 6, infection n = 16, malignancy n = 11). Moreover, only patients who received an HLH associated ICD-10 code could be included in the study. Since HLH is thought to be an underdiagnosed syndrome [60], the number of unreported cases could have been substantial. In addition, it seems plausible that a selection bias towards patients with fatal outcome or refractory disease, who did not fulfill diagnostic criteria or did not respond to therapy for more common diseases such as sepsis and malignancies, occurred. Finally, with four hematologic/oncologic hospitals cooperating, a selection bias towards patients with malignancy associated HLH could not be avoided. [2]

5. Conclusion

HLH is a rare but often fatal hyperinflammatory syndrome primarily appearing alongside malignancies, infections, autoimmune diseases, and genetic mutations. The aim of this study was to collect and analyze data concerning epidemiology, diagnostic and therapeutic measurements, clinical outcome, and prognosis of adult HLH patients in order to help improving their clinical management. Therefore, a multicenter retrospective study was conducted. The inclusion of 62 patients of four hematologic/oncologic clinics, should have allowed the description of the majority of adult HLH patients in the metropolitan area of Munich treated over the last years. [2]

The epidemiologic and clinical data of the population was generally in accordance with previous publications. With regard to the dismal prognosis of patients with malignancy associated HLH – the one-year survival probability was 21% and overall mortality reached 77% – further progress in early diagnosis and therapies is needed in order to improve the outcome of affected individuals.

A possible adverse prognostic parameter for detecting patients with a poor prognosis may be sCD25, which was the only significant covariate in the multivariate Cox-regression model ($p = 0.005$). Thus, it could play a central role in the clinical decision-making, for example regarding the early administration of an etoposide containing regimen. The impact of this finding appears even more important when considering the limited availability of sCD25 in previous studies. Nevertheless, one has to take into account the possibility of a long laboratory turn-around time of sCD25 in the clinical setting, prolonging the diagnostic and prognostic work-up. Therefore, a broad availability of an emergency assessment in the clinical laboratories of tertiary care hospitals is desirable.

Regarding diagnostic criteria, all patients presented with elevated serum ferritin levels > 500 µg/L. However, an increased ferritin cutoff in the adult population seems reasonable in order to increase the sensitivity of this parameter. Finally, HLH diagnosis should be questioned in individuals without ferritin levels exceeding 500 µg/L. Even though, ferritin values at treatment initiation did not show a prognostic correlation concerning overall survival in the univariate Cox-regression model, they may serve as a valuable, broadly available, and cost-effective parameter for assessing the course of the HLH episode.

The use of metabolic imaging such as PET-CT did not allow the detection of an underlying malignant or infectious trigger. The positive findings were also rather unspecific. However, with regard to previous studies, these elaborate imaging techniques may be of

diagnostic and prognostic benefit in patients with unknown underlying triggers, especially when the suspicion of lymphoma arises.

Despite major progress being made in the last years concerning a better understanding of the pathophysiology, and improvements in standardizing diagnosis and treatment, HLH remains a critical clinical syndrome with plenty of room for further research and ameliorations.

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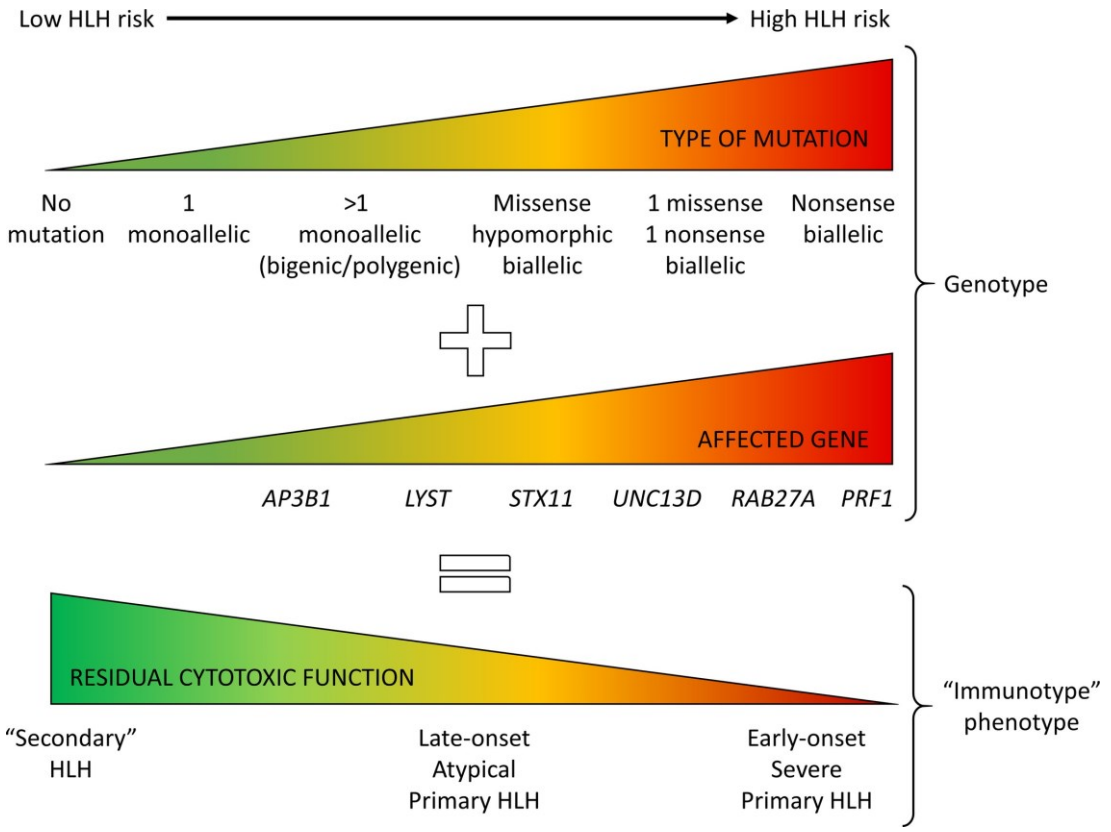
Supplement

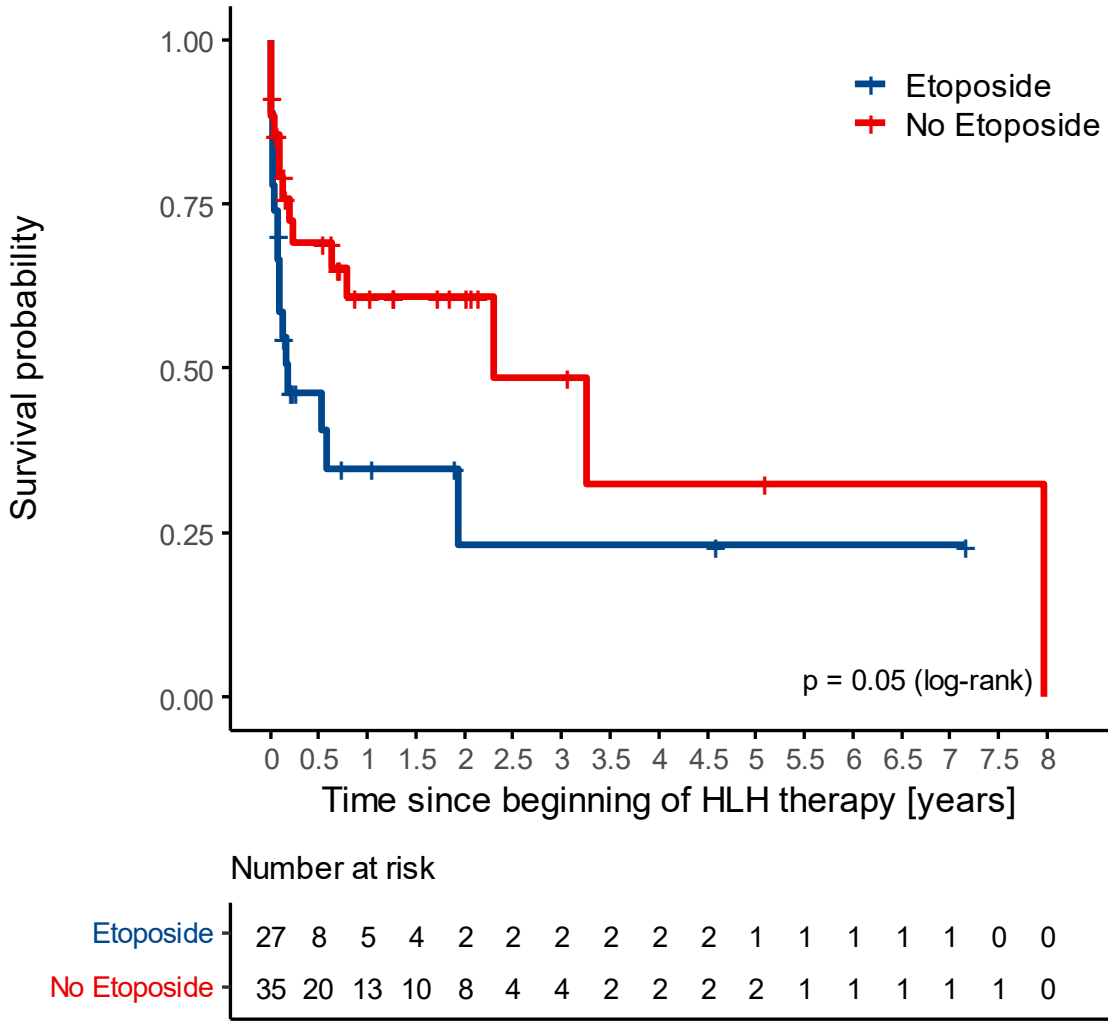
Website HScore:

https://www.mdcalc.com/hscore-reactive-hemophagocytic-syndrome

Retrieved August 07 2023.



Supplemental Figure 1 Effect of mutation on HLH onset. (Brisse, Wouters, and Matthys, *Advances in the pathogenesis of primary and secondary haemophagocytic lymphohistiocytosis: differences and similarities* © 2016 John Wiley and Sons [6], license acquired via *Copyright Clearance Center* (license number 5607050279962)).



Supplemental Figure 2 Kaplan-Meier curve comparing etoposide treatment. The significant difference in survival probability is likely due to a selection bias: more severe and refractory cases have possibly received an intensified treatment scheme, e.g., with etoposide.

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Affidavit

Wimmer, Thomas

Surname, first name

I hereby declare, that the submitted thesis entitled

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is my own work. I have only used the sources indicated and have not made unauthorised use of services of a third party. Where the work of others has been quoted or reproduced, the source is always given.

I further declare that the dissertation presented here has not been submitted in the same or similar form to any other institution for the purpose of obtaining an academic degree.

Munich, 07/23/2024

Place, Date

Thomas Wimmer

Signature doctoral candidate

List of publications

Parts of this dissertation have been published in:

1. Wimmer, T., et al., *Auswertung erwachsener Patienten mit hämophagozytischer Lymphohistiozytose (HLH): eine retrospektive Analyse* [Poster Presentation]. Jahrestagung der Deutschen, Österreichischen und Schweizerischen Gesellschaften für Hämatologie und Medizinische Onkologie, 7.–10. Oktober 2022, Wien: Abstracts. Oncology Research and Treatment, 2022. **45(suppl 2)**: p. 82.

2. Wimmer, T., et al., *sCD25 as an independent adverse prognostic factor in adult patients with HLH: results of a multicenter retrospective study*. Blood Adv, 2023. **7**(5): p. 832-844.