bjh review

Understanding the spectrum of haemophagocytic lymphohistiocytosis: update on diagnostic challenges and therapeutic options

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Summary

The cytokine storm syndrome 'haemophagocytic lymphohistiocytosis' (HLH) is an under-recognized hyperinflammatory disorder, causing high morbidity and mortality risk in children and adults. It can be subdivided into a primary, genetic form and a secondary, acquired form that complicates diverse infections, malignancies and autoimmune or autoinflammatory disorders. Both subtypes present with the same spectrum of non-specific symptoms, making accurate diagnosis and rapid treatment initiation challenging. In the last decade, increased awareness and international collaborative efforts fuelled a marked progress in diagnostic protocols and novel treatment strategies for HLH and new diagnostic guidelines are being tailored to specific secondary HLH subtypes. Therapy is gradually shifting its focus from overall immunosuppression towards targeting specific cytokines, cell types or signalling pathways underlying pathophysiology. Nevertheless, continued research efforts remain indispensable to customize therapy to individual patient needs.

Keywords: haemophagocytic syndrome, haemophagocytic lymphohistiocytosis, macrophage activation syndrome, diagnosis, treatment.

Haemophagocytic lymphohistiocytosis (HLH) was first described in 1939 as 'histiocytic medullary reticulocytosis' (Scott & Robb-Smith, 1939) and has since been recognized to comprise a heterogeneous spectrum of clinically similar but aetiologically diverse subtypes, affecting all ages. Based on the underlying aetiologies, HLH is subdivided into primary and secondary forms (Table I). Primary HLH is caused by mutations in genes regulating granule-dependent cytotoxicity of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells. Mutations in nine genes have been linked to primary HLH development, creating further subdivision into familial HLH (FHL) type 1 to 5 and HLH associated with Griscelli syndrome type 2 (GS2), Chédiak–Higashi syndrome (CHS), Hermansky–Pudlak syndrome type 2 (HPS2) and X-linked lymphoproliferative disease (XLP) types 1 and 2. Primary HLH may also complicate inborn metabolic disorders such as lysinuric protein intolerance (Janka & Lehmberg, 2014). Secondary HLH is not inherited but complicates various medical conditions. Infection-associated secondary HLH, predominantly triggered by viruses like Epstein-Barr virus (EBV) and cytomegalovirus, is most frequently observed (Ramos-Casals *et al*, 2014). Secondary HLH also occurs in malignancies and different autoimmune or autoinflammatory diseases, such as systemic juvenile idiopathic arthritis (sJIA), systemic lupus erythematosus (SLE) and adult-onset Still disease (AOSD). In the latter cases, it is often called 'macrophage activation syndrome' (MAS). Recently, involvement of heterozygous mutations in MAS and other secondary HLH subtypes has been reported, indicating possible genetic predisposition to secondary HLH and blurring the distinction with primary HLH (Zhang *et al*, 2011, 2014).

The incidence of HLH has been estimated at 1·2 per million children per year in Europe and Japan (Aricò *et al*, 2001; Ishii *et al*, 2007; Meeths *et al*, 2015). In the USA, a prevalence of 1 in 100 000 was calculated (Niece *et al*, 2010). These numbers will probably increase in the future due to improved genetic screening methods and more specific diagnostic criteria for different secondary HLH subtypes, increasing recognition. The survival of HLH patients has significantly improved due to advances in therapeutic protocols (Meeths *et al*, 2015). Nonetheless, HLH remains associated with high morbidity and mortality of approximately 20-40% (Niece *et al*, 2010; Cetica *et al*, 2016), which can rise up to 70–85% in certain subtypes (Ishii *et al*, 2007; Rouphael *et al*, 2007).

Spectrum of disease symptoms in HLH

Both primary and secondary HLH are characterized by a wide spectrum of non-specific inflammatory symptoms,

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Table I. Classification of primary and secondary HLH.

Primary HLH			Secondary HLH
Subtype	Mutation	Protein	Associated with
Familial HLH type 1	Unknown	Unknown	Infections
Familial HLH type 2	*PRF1*	Perforin	Viral (EBV, CMV, etc.)
Familial HLH type 3	*UNC13D*	Munc13-4	Bacterial (*Mycobacterium*, etc.)
Familial HLH type 4	*STX11*	Syntaxin-11	Fungal (*Histoplasma,* etc.)
Familial HLH type 5	*STXBP2*	Munc18-2	Parasitic (*Leishmania,* etc.)
Griscelli syndrome type 2	*RAB27A*	Rab27a	Malignancy
Chédiak–Higashi syndrome	*LYST*	LYST	(Lymphoma, leukaemia, etc.)
Hermansky–Pudlak syndrome type 2	*AP3B1*	β3A of AP3	Autoimmune/autoinflammatory diseases
X-linked lymphoproliferative disease			"Macrophage activation syndrome"
Type 1	*SH2D1A*	SAP	(sJIA, SLE, Kawasaki disease, etc.)
Type 2	*XIAP*	XIAP	

β3A of AP3, β3A subunit of adaptor protein 3; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HLH, haemophagocytic lymphohistiocytosis; LYST, lysosomal trafficking regulator; SAP, SLAM-associated protein; sJIA, systemic juvenile idiopathic arthritis; SLAM, signalling lymphocyte activation molecule; SLE, systemic lupus erythematosus; XIAP, X-linked inhibitor of apoptosis.

making early diagnosis challenging. Not every patient will develop all characteristics and some features might be absent upon initial disease presentation.

Haemophagocytosis

Haemophagocytic lymphohistiocytosis derives its name from the pathological finding of haemophagocytosis, in which activated macrophages engulf blood cells or their precursors. This phenomenon is not always present at disease presentation, but can often be detected upon repeated examinations of cerebrospinal fluid. Haemophagocytes infiltrate several organs, including spleen, liver, lymph nodes, heart, pancreas and meninges (Grom, 2004; Schaer *et al*, 2005; Avcin *et al*, 2006). Haemophagocytosis has also been observed in other hyperinflammatory diseases, such as sepsis, systemic inflammatory response syndrome (SIRS), multi-organ dysfunction syndrome (MODS), influenza, leishmaniasis and malaria (Castillo & Carcillo, 2009; Zoller *et al*, 2011). Thus, it is no pathognomonic feature of HLH, and the number of haemophagocytes in bone marrow aspirates correlates poorly with the probability of HLH development (Weaver & Behrens, 2014). Furthermore, it is not clear whether haemophagocytosis plays a pathogenic, bystander or immunoregulatory role in HLH. Data in murine HLH designate the haemophagocytes as important mediators of anaemia and, possibly, of leucopenia, neutropenia and thrombocytopenia, through a direct γ-interferon (IFNγ)-stimulated process of consumptive macropinocytosis (Zoller *et al*, 2011). Increased cytokine levels in HLH patients are reported to induce down-regulation of the anti-phagocytic CD47 protein on haematopoietic stem cells, thus provoking engulfment by macrophages and the development of bone marrow hypocellularity (Kuriyama *et al*, 2012). Conversely, an immunoregulatory role for haemophagocytosis was deduced from the observation that haemophagocytes in human and murine HLH express CD163, a marker present on alternatively activated macrophages, and a scavenging receptor that internalizes haemoglobin-haptoglobin complexes, reducing the amount of free haem and limiting oxidative stress (Schaer *et al*, 2005; McCoy *et al*, 2012; Canna *et al*, 2014). Hence, CD163 upregulation can be seen as a regulatory mechanism preventing tissue damage during excessive inflammation. Levels of soluble CD163 (sCD163) are also elevated in patients, representing a marker for the degree of macrophage activation and haemophagocytosis (Schaer *et al*, 2005; Avcin *et al*, 2006).

Clinical symptoms

Haemophagocytic lymphohistiocytosis is characterized by persistent high fever, a bleeding diathesis and hepatosplenomegaly with lymphadenopathy, both indicative of lymphoproliferation. Patients display progressive hepatic dysfunction with jaundice and ultimately develop multi-organ failure. Skin rash can occur in several forms, often erythematous or purpuric. Neurological involvement is present in 25–50% of patients, ranging from irritability and headaches to ataxia, encephalopathy, seizures and coma (Horne *et al*, 2008; Ramos-Casals *et al*, 2014).

Laboratory abnormalities

Haemophagocytic lymphohistiocytosis patients develop severe cytopenias, predominantly anaemia and thrombocytopenia, and in the later stages, progressive neutropenia. The cause remains speculative. Elevated levels of pro-inflammatory cytokines can depress haematopoiesis, however, bone marrow aspirates of patients have been reported to be hypocellular, normocellular or even hypercellular during cytopenic episodes (Jordan *et al*, 2011). Considering the acute drop in cell numbers, a consumptive aetiology has

been proposed, in which haemophagocytosis may play a role (Zoller *et al*, 2011; Kuriyama *et al*, 2012). Thrombocytopenia can also result from active disseminated intravascular coagulation (DIC). HLH patients often develop a coagulopathy resembling DIC, with elevated D-dimers and hypofibrinogenaemia (Jordan *et al*, 2011; Valade *et al*, 2015). Hypofibrinogenaemia can also be attributed to increased secretion of plasminogen activator by activated macrophages, or might be secondary to decreased fibrinogen production by a distressed liver. Liver dysfunction in HLH is reflected in elevated levels of hepatic enzymes, such as alanine transaminase (ALT), aspartate transaminase (AST) and lactate dehydrogenase (LDH). Levels of triglycerides and bilirubin can be mildly to highly elevated (Grom, 2004).

Elevated levels of soluble CD25 (sCD25) and soluble CD8 reflect excessive T cell activation in HLH (Akashi *et al*, 1994). Soluble Fas and Fas-ligand can be increased, which may interfere with the process of activation-induced cell death and prolong survival of activated immune cells (Emmenegger *et al*, 2000). A striking hyperferritinaemia is a most typical feature of HLH. A study comparing aetiologies of 'ultrahyperferritinaemia' revealed HLH as the entity with the highest mean ferritin levels, exceeding 20 000 ng/ml (Beer & Vadakara, 2015). Along with elevated sCD163 and neopterin (Ibarra *et al*, 2011), ferritin is considered a marker of macrophage activation in HLH. It is an acute phase protein, consisting of H- and L-subunits, binding free iron to prevent oxidative damage. H-subunit rich ferritin is predominantly produced by macrophages, and varies with cytokine levels, whereas L-subunit rich ferritin is abundantly present in the liver and fluctuates little during inflammation. In healthy individuals, plasma ferritin is mainly glycosylated, whereas injured or necrotic tissue releases non-glycosylated ferritin (Koorts & Viljoen, 2011). In HLH the percentage of glycosylated ferritin is surprisingly low (±20%), indicating tissue damage. Although both H- and L-ferritin are elevated in patients, the increase is more pronounced for H-ferritin, resulting in an imbalance. This imbalance, and the glycosylation percentage, have been proposed as diagnostic markers for HLH (Wang *et al*, 2009; Ruscitti *et al*, 2015).

Lastly, CTL and NK cell cytotoxicity are inherently defective in primary HLH. An acquired NK cell defect is present in approximately 20% of secondary HLH patients, which typically normalizes upon disease remission or after *in vitro* stimulation with interleukin 2 (IL2). Decreased NK cell numbers and/or reduced perforin expression can form the basis of the secondary cytotoxicity defect (Grom, 2004; Bryceson *et al*, 2012).

Cytokine storm

Cytokines have been reported to rise to higher levels in primary and secondary HLH, compared to other inflammatory disorders like infectious mononucleosis or lymphoma (Wada *et al*, 2013; Maruoka *et al*, 2014). Levels of IFNγ, IL1β, IL6, IL12, IL18 and tumour necrosis factor-α (TNFα, also termed TNF) are frequently elevated in HLH (Akashi *et al*, 1994; Osugi *et al*, 1997; Mazodier *et al*, 2005; Kuriyama *et al*, 2012; Put *et al*, 2015). Levels of megakaryocyte colony-stimulating factor (M–CSF), granulocyte-macrophage colony-stimulating factor (GM)–CSF and IL27 may also be increased (Akashi *et al*, 1994; Nold-Petry *et al*, 2010; Kuriyama *et al*, 2012), along with chemokines like IL8/CXCL8, MIG/CXCL9, IP10/CXCL10, I–TAC/CXCL11, MCP–1/CCL2, MIP–1α/CCL3 and MIP–1β/CCL4 (Teruya-Feldstein *et al*, 1999; Tamura *et al*, 2008; Bracaglia *et al*, 2015; Put *et al*, 2015). These cytokines and chemokines stimulate tissue infiltration and activation of immune cells, driving on-going cytokine production that culminates into a cytokine storm. In response, levels of anti-inflammatory IL10 and IL18-binding protein (IL18BP) are also increased, but may not be sufficient to temper the overwhelming immune activation (Osugi *et al*, 1997; Mazodier *et al*, 2005). A discrepancy between the increase in IL18 and its antagonist IL18BP has been reported, resulting in aberrantly high levels of free IL18 (Mazodier *et al*, 2005). The imbalance was explained in one patient by reduced induction of IL18BP following IFNγ stimulation, revealing a failure of this negative feedback mechanism (Nold-Petry *et al*, 2010). Data in murine HLH, showing the efficacy of recombinant IL18BP treatment, corroborate the pathogenic role of excess free IL18 in HLH (Chiossone *et al*, 2012).

Higher cytokine levels are correlated with poor prognosis in patients (Fisman, 2000). The cytokine storm is thought to elicit cardinal features of HLH. Fever can be induced by increased levels of IL1, IL6 and TNFα (Janka, 2007). These cytokines, and IFNγ, can also increase ferritin production (Koorts & Viljoen, 2011). IL1β, IFNγ and TNFα have the potency to suppress haematopoiesis and thus contribute to the development of cytopenia. IL6 can also mediate anaemia. Additionally, overexpression of IL6 down-modulates NK cell cytotoxicity by reducing perforin and granzyme B protein levels (Cifaldi *et al*, 2015). High concentrations of TNFα induce hypertriglyceridaemia by stimulating triglyceride synthesis and inhibiting the function of lipoprotein lipase (Grom, 2004). Coagulopathy can be related to increased levels of IL1β, which activates plasminogen to induce fibrinolysis, while DIC can develop as a result of IFNγ and TNFα overproduction. The latter two also mediate liver damage (Créput *et al*, 2008). Lastly, IFNγ is considered a key mediator of haemophagocytosis (Zoller *et al*, 2011). Persistent overexpression of IFNγ in the 'Yeti' mouse model provoked spontaneous splenomegaly, lymphadenopathy, hyperferritinaemia, liver necrosis and macrophage activation, partially resembling HLH (Reinhardt *et al*, 2015).

Distinguishing features of primary and secondary HLH

Given that primary and secondary HLH are highly similar in phenotype, and are both predominantly triggered by

infections, differentiating these subtypes at presentation is challenging. Nonetheless, it is essential for rapid indication of the necessity for allogeneic haematopoietic stem cell transplantation (HSCT), selection of a suitable family donor and (prenatal) screening of siblings in affected families (Cetica *et al*, 2016). Accurate classification of different HLH subtypes is also essential in research and cohort design to identify optimal diagnostic algorithms and therapy for distinct patient groups (Canna & Behrens, 2012).

Classic dissimilarities between primary and secondary HLH comprise the age at disease onset and disease severity. Primary HLH typically develops in the first years of life; the median age is usually higher in secondary HLH. Infection- and autoimmunity/autoinflammation-associated HLH predominantly occur in children and adolescents, while malignancy-associated HLH is most common in adults and the elderly (Ishii *et al*, 2007). Prognosis is generally worse in primary HLH, with mortalities around 50%, compared to 10–15% in secondary HLH (Cetica *et al*, 2016).

Cytokine profiles may also differ. It has been suggested that each HLH subtype carries a cytokine signature reflective of the underlying aetiology. For instance, levels of IL1β are often normal in primary HLH, but elevated in secondary HLH (Janka, 2007; Put *et al*, 2015). In autoinflammation/autoimmunity-associated HLH, IL18 appears to be higher in sJIA- and AOSD-associated HLH, while M-CSF is most increased in SLE-associated HLH (Maruyama & Inokuma, 2010; Shimizu *et al*, 2010). IL18 levels are also significantly higher in sJIA-associated HLH compared to EBV-induced HLH (Shimizu *et al*, 2010). Cytokine profiles also differ between B-cell or T/NK-cell lymphoma-associated HLH (Maruoka *et al*, 2014).

The frequency and severity of clinical symptoms or laboratory abnormalities can also vary between primary and secondary HLH. Hypopigmentation and albinism occur almost exclusively in CHS-, GS2- or HPS2-associated primary HLH (Janka & Lehmberg, 2014). Severe central nervous system (CNS) involvement may also be more common in primary HLH (Horne *et al*, 2008). Fibrinogen levels were lower in FHL when compared to infection-associated HLH, notwithstanding high inter-patient variability (Bode *et al*, 2015). Bilirubin increased to higher levels in primary HLH, while C-reactive protein (CRP) levels were higher in secondary HLH (Ozen *et al*, 2014). Yasumi *et al* (2015) combined different parameters to distinguish paediatric FHL from EBV–, herpes simplex virus- or sJIA-related secondary HLH. The total lymphocyte percentage and sCD25 levels in peripheral blood were higher, while the concentration of LDH and ferritin was lower, resulting in increased sCD25/ferritin ratios in FHL compared to the secondary HLH subtypes. In contrast, higher sCD25/ferritin ratios were also reported for lymphoma-associated secondary HLH, a subgroup not included in this study (Yasumi *et al*, 2015). Lehmberg *et al* (2013) were able to distinguish patients with sJIA-associated HLH from FHL and virus-induced HLH on the basis of higher CRP and neutrophil counts, together with lower sCD25 levels. In secondary HLH, symptoms may reflect the underlying inflammatory disorder or vary with the inducing factor. In HLH complicating sJIA or AOSD, platelet, leucocyte and neutrophil counts, as well as fibrinogen levels are generally higher than in primary HLH (Lehmberg *et al*, 2013). In EBV-induced secondary HLH, levels of ferritin, AST, ALT and LDH were higher in comparison with non-EBV-related HLH (Chen *et al*, 2016).

Several proteins can possibly be used as biomarkers to differentiate HLH subtypes. The alarmin S100A12, an endogenous monocyte-activating Toll-like receptor (TLR)-4 ligand, can be used to distinguish sJIA-associated HLH. Median S100A12 serum levels in sJIA-associated HLH were over 12 times higher than in primary HLH or other secondary HLH subtypes (Holzinger *et al*, 2015). Neopterin, secreted by activated macrophages and dendritic cells, appeared to be lower in sJIA-associated HLH, compared to EBV-induced HLH (Shimizu *et al*, 2010).

In conclusion, different cohorts have identified distinguishing features between primary and secondary HLH (Table II) or different secondary HLH subtypes. Nonetheless, some caveats are in place. Not all studies report similar findings. High inter-patient variability might obscure differences and reported dissimilarities may be temporal or related to the stage of disease progression (Yasumi *et al*, 2015). Underlying disease activity, concomitant patient treatment and measuring techniques also vary between different studies, complicating the interpretation of these findings. Larger

Table II. Possible distinguishing factors between primary and secondary HLH.

Factors differentiating primary from secondary HLH
Lower age at disease presentation (Cetica *et al*, 2016)
More frequent albinism/hypopigmentation (Janka & Lehmberg, 2014)
More frequent or severe CNS involvement (Horne *et al*, 2008)
Lower IL-1β levels (Janka, 2007; Put *et al*, 2015)
Lower fibrinogen levels (Lehmberg *et al*, 2013; Bode *et al*, 2015)
Higher bilirubin levels (Ozen *et al*, 2014)
Lower CRP (Lehmberg *et al*, 2013; Ozen *et al*, 2014)
<-->No difference in CRP (Yasumi *et al*, 2015)
Higher total lymphocyte percentage (Yasumi *et al*, 2015)
<-->Lower WBC, platelets, neutrophil counts (Lehmberg *et al*, 2013)
Higher sCD25 levels (Lehmberg *et al*, 2013; Bode *et al*, 2015; Yasumi *et al*, 2015)
Lower ferritin levels (Yasumi *et al*, 2015)
<-->No difference in ferritin (Ozen *et al*, 2014)
Higher sCD25/ferritin ratio (Yasumi *et al*, 2015)
Lower LDH levels (Yasumi *et al*, 2015)
Lower S100A12 levels (Holzinger *et al*, 2015)

CNS, central nervous system; CRP, C-reactive protein; HLH, haemophagocytic lymphohistiocytosis; LDH, lactate dehydrogenase; sCD25, soluble CD25; WBC, white blood cells.

cohorts should provide confirmation before these parameters can be used in differential diagnosis of primary versus secondary HLH or to predict underlying aetiologies in secondary HLH.

Diagnosis of HLH

Prompt diagnosis of HLH is challenging because its clinical picture is non-specific. HLH features overlap with symptoms observed in disseminated infections, haematological malignancies and other cytokine storm syndromes like SIRS and MODS (Castillo & Carcillo, 2009; Canna & Behrens, 2012).

HLH-2004 criteria

To facilitate and standardize the diagnosis of HLH, the Histiocyte Society developed a set of guidelines that are combined to increase specificity for HLH. The original criteria of 1991 were revised in 2004 (Henter *et al*, 2007). A clinical diagnosis of HLH requires at least 5 of the 8 HLH-2004 criteria to be fulfilled (Table III). Confirmation of a molecular defect consistent with primary HLH also suffices. Flow cytometry can give guidance on which genes should be sequenced: when intracellular perforin, SAP or XIAP staining is low, mutations in *PRF1, SH2D1A* or *XIAP,* respectively, need to be considered. When CD107a surface expression is reduced or absent, *UNC13D, STX11, STXBP2, RAB27A, LYST* and *AP3B1* analyses should be performed because the patient possesses a defect in the degranulation pathway. The latter three genes should especially be explored when hypopigmentation is present, which is detectable via hair microscopy (Bryceson *et al*, 2012; Janka & Lehmberg, 2014).

Although the HLH-2004 criteria (Henter *et al*, 2007) have allowed great progress in the diagnosis of HLH, some limitations need to be addressed. Often, not all criteria are fulfilled at disease presentation, which could misguide physicians towards a negative diagnosis. Symptoms like neutropenia, consumptive hypofibrinogenaemia and elevated D-dimers typically arise later (Janka & Lehmberg, 2014). Therefore, diagnosis should not rely exclusively on absolute values. The dynamic pattern of certain parameters indicates development of particularly secondary HLH, i.e. falling erythrocyte sedimentation rate (ESR), fibrinogen level and leucocyte or platelet count (Weaver & Behrens, 2014). Based on expert consensus and confirmed on real patient data, decreases in platelet counts and elevations of ferritin and AST were considered the most valuable changes over time for early diagnosis of sJIA-associated HLH (Ravelli *et al*, 2016a). Hence, general cut-offs for the diagnosis of all HLH subtypes are difficult to define, rather, dynamic changes should be integrated into the diagnostic protocol. Currently, the ferritin cut-off is a matter of debate. To further increase the specificity of the HLH-2004 criteria, raising the ferritin cut-off from 500 μg/l up to >2000 μg/l has been suggested. As malignancies also present with increased ferritin levels, this cut-off is particularly decisive in adult HLH where the higher incidence of malignancies may confound diagnosis (Lehmberg *et al*, 2014; Saeed *et al*, 2015). Lastly, the criterion of haemophagocytosis appears to have a limited diagnostic value, as it is neither specific for HLH, nor a sensitive measurement. As haemophagocytosis requires an invasive sampling procedure and correlates poorly with disease severity, its exclusion from the diagnostic criteria has been proposed (Weaver & Behrens, 2014; Cetica *et al*, 2016). However, when bone marrow aspiration is performed to exclude underlying malignancies, a search for haemophagocytes may be undertaken to confirm diagnosis.

Specific secondary HLH criteria

Although the HLH-2004 diagnostic criteria were theoretically proposed for both primary and secondary HLH, in practice they may perform suboptimal for certain secondary HLH subtypes. Therefore, several groups have proposed distinct diagnostic guidelines. A numerical score, called the HScore, was developed based on a retrospective multicentre cohort study (Fardet *et al*, 2014). It is calculated in an online scoring system, based on 9 clinical, laboratory and histological variables, to estimate the risk of secondary HLH (Table III). Different weights are assigned to the criteria, to accumulate into a score ranging from 0 to 337, corresponding to the probability of HLH development. A cut-off of 169 is estimated to correctly identify 90% of secondary HLH patients (Fardet *et al*, 2014).

Nevertheless, distinguishing secondary HLH from the underlying inflammatory disease remains challenging, as several key symptoms may already be present in the underlying disorder. Therefore, specific criteria have been proposed to diagnose HLH complicating sJIA, SLE or malignancies.

In 2005, the first preliminary diagnostic guidelines for HLH in sJIA were published (Ravelli *et al*, 2005). Relative changes in disease status, rather than absolute cut-offs, were emphasized (Table III). Retrospective evaluation of the preliminary criteria in 2014 determined that they outperformed the HLH-2004 criteria in distinguishing sJIA-associated HLH from active sJIA. Addition of hyperferritinaemia enhanced their ability to differentiate sJIA-related HLH from confounding systemic infections (Davì *et al*, 2014). In 2015, new classification criteria for sJIA-related HLH were launched (Ravelli *et al*, 2016b). Paediatric rheumatologists worldwide were asked to reflect on the diagnostic value of different parameters and to select those most suited for detecting HLH in sJIA. This survey produced 9 candidate parameters (Table III) (Davì *et al*, 2011), that were validated using patient data, comparing their diagnostic performance using either absolute cut-offs or relative changes. The final, renewed diagnostic guidelines for sJIA-associated HLH were established in an international expert conference (Table III) (Ravelli *et al*, 2016b). Remarkably, the cut-off values for the platelet count and fibrinogen level were within the normal

Table III. Diagnostic criteria for primary and secondary HLH

	Diagnostic criteria for HLH							
	Primary HLH	Secondary HLH						
	HLH-2004 criteria (Henter *et al*, 2007)	HScore (Fardet *et al*, 2014)	Preliminary diagnostic guidelines for HLH in sJIA (Ravelli *et al*, 2005)	Expert survey for HLH in sJIA (Davi *et al*, 2011)	Consensus classification criteria for HLH in sJIA (Ravelli *et al*, 2016)	Preliminary diagnostic criteria for early HLH in sJIA (Kostik *et al*, 2015)	Preliminary diagnostic criteria for HLH in SLE (Parodi *et al*, 2009)	Diagnostic criteria for lymphoma-associated HLH (Shimazaki et al, 2000)
Clinical criteria								
Underlying immunodepression		+						
Fever	+	+		+	+		> 38°C	≥ 1 week ≥ 38.5 °C
Hepatomegaly		+	+				≥ 3cm below costal arch	+ [e]
Splenomegaly	+	+					≥ 3cm below costal arch	+
Haemorrhages			+				+	
CNS dysfunction			+				+	
Laboratory criteria								
Cytopenia in ≥ 2 blood cell lineages	+ [a]						+ [c]	
Hemoglobin	< 90 g/liter [a]	+					≤ 90 g/liter [c]	≤ 90 g/liter [d]
Platelets	< 100 x 10^9/liter [a]	+	+	+	≤ 181 x 10^9/liter	≤ 211 x 10^9/liter	≤ 150 x 10^9/liter [c]	< 100 x 10^9/liter [d]
Neutrophils	< 1 x 10^9/liter [a]							
WBC		+	+	+		≤ 9·9 x 10^9/liter	≤ 4 x 10^9/liter [c]	
Hypertriglyceridaemia	≥ 3.0 mmol/liter [b]	+		+	> 1·8 mmol/liter		> 2·0 mmol/liter	
Hypofibrinogenaemia	≤ 1.5 g/liter [b]	+	+	+	≤ 3·6 g/liter	≤ 1·8 g/liter	≤ 1·5 g/liter	
Elevated fibrin degradation products								+ [e]
Hyperferritinaemia	≥ 500 µg/liter	+		+	> 684 µg/liter	> 400 µg/liter	> 500 µg/liter	≥ 10 µg/liter [e]
Elevated LDH						> 822 U/liter	> 567 U/liter	≥ 2 x standard upper limit [e]
Elevated liver enzymes		Increased AST	Increased AST	+	AST > 48 U/liter	AST > 59·7 U/liter	AST > 40 U/liter	
Falling ESR				+				
Low or absent NK cell activity	+							
Elevated sCD25	≥ 2400 U/ml							
Hypoalbuminaemia						< 29 g/liter		
Proteinuria						+		
Histopathologic criterion								
Haemophagocytosis	+	+	(*)	+			(*)	+
Diagnostic key:	Diagnosis requires either a molecular diagnosis of primary HLH or presence of ≥ 5 of 8 diagnostic criteria. For [a] ≥ 2 subitems required. For [b] ≥ 1 subitem required. No evidence of malignancy.	Online weighted score from 0 to 337 with 169 corresponding to 90% probability of HLH development.	Diagnosis requires presence of ≥ 2 laboratory and/or clinical criteria.	Candidate parameters for inclusion in diagnostic criteria.	Mandatory criteria: known or suspected sJIA, fever and increased ferritin, combined with ≥ 2 of the remaining laboratory criteria.	Diagnosis requires presence of ≥ 3 laboratory criteria.	Diagnosis requires presence of ≥ 1 clinical criterion and ≥ 2 laboratory criteria. For [c] ≥ 2 subitems are required.	Diagnosis requires all criteria to be fulfilled. For [d] ≥ 1 subitem should be fulfilled, for [e] ≥ 2 subitems should be fulfilled. No evidence of infection. Histopathologically confirmed malignant lymphoma.

AST, aspartate transaminase; CNS, central nervous system; ESR, erythrocyte sedimentation rate; HLH, haemophagocytic lymphohistiocytosis; LDH, lactate dehydrogenase; NK, natural killer; sCD25, soluble CD25; sJIA, systemic juvenile idiopathic arthritis; SLE, systemic lupus erythematosus; U, unit; WBC, white blood cells; + represents a diagnostic criterion of which no threshold value is specified; (*) A bone marrow aspirate is only required in doubtful cases.

laboratory range and thus notably higher than the corresponding thresholds in the HLH-2004 criteria. A relative decrease is sufficient to raise suspicion of HLH in sJIA patients with otherwise characteristically elevated fibrinogen and thrombocytosis (Lehmberg *et al*, 2013; Ravelli *et al*, 2016b). A smaller study (Kostik *et al*, 2015) proposed a slightly different set of 8 preliminary guidelines to ensure early recognition of HLH in sJIA (Table III). Similar to the criteria established by Ravelli *et al* (2016b), the platelet cut-off was within the normal laboratory range, while the threshold for hypofibrinogenaemia was considerably lower. The cut-off for hyperferritinaemia was unexpectedly low, probably due to the study's focus on detecting early laboratory changes in HLH development (Table III) (Kostik *et al*, 2015).

To facilitate the recognition of HLH in SLE patients, preliminary guidelines were selected that discriminated SLE-associated HLH from active SLE with the greatest sensitivity and specificity (Table III). As cytopenias are frequently present in juvenile SLE, lower cut-off values for blood cell counts were chosen as compared to the diagnostic criteria for HLH in sJIA (Parodi *et al*, 2009).

Currently, no generally accepted guidelines for the identification of malignancy-associated HLH exist. In 1999, a Japanese group proposed diagnostic criteria for adult lymphoma-associated HLH (Table III; Takahashi *et al*, 1999; Shimazaki *et al*, 2000), but these did not achieve wide recognition. Moreover, the requirement for no concomitant infections is no longer supported in current literature (Shimazaki *et al*, 2000). To distinguish HLH-specific features from neoplasm-related features, the Histiocyte Society recently reported consensus recommendations for the diagnosis of malignancy-associated secondary HLH (Lehmberg *et al*, 2015). A subdivision was made between HLH occurring at the presentation or relapse of malignancy and HLH occurring during chemotherapeutic treatment for a malignant condition. For both subgroups, a diagnostic flow chart was created, primarily based on the HLH-2004 criteria. The flow chart also aides the exclusion of occult malignancies in suspected HLH patients (Lehmberg *et al*, 2015).

Biomarkers

Numerous biomarkers have been proposed to facilitate early recognition of HLH. An increased H-ferritin/L-ferritin ratio (Ruscitti *et al*, 2015), low percentage of glycosylated ferritin (Wang *et al*, 2009), an elevated ferritin/ESR ratio (Gorelik *et al*, 2013), high levels of the alarmin HMGB1 and its oxidation status (Palmblad *et al*, 2014), increased levels of neopterin (Ibarra *et al*, 2011), elevated sCD163 (Schaer *et al*, 2005), high serum and urine β2-microglobulin levels (Hibi *et al*, 1995), increased serum levels of follistatin-like protein-1 (Gorelik *et al*, 2013) and heightened sphingomyelinase activity (Jenkins *et al*, 2013) are all indicative of HLH development. Alterations in cytokine or chemokine balances may also possess prognostic value in HLH. A decrease in the IL18/IFNγ ratio or high IL18 serum levels can predict HLH development in sJIA (Shimizu *et al*, 2010, 2015; Put *et al*, 2015), while high levels of CXCL9 and CXCL10 represent biomarkers for the diagnosis of lymphoma-associated HLH and reflect treatment outcome. Levels of CXCL9 and CXCL10 also mirror disease severity in MAS (Maruoka *et al*, 2014; Bracaglia *et al*, 2015). Recently, increased expression of different microRNAs has been associated with HLH (Bay *et al*, 2013), while levels of EBV-encoded microRNA-BART-16-1 show prognostic value in EBV-associated HLH (Zhou *et al*, 2015). Lastly, downregulation of CD5 expression on $CD8^+$ T cells has been proposed as a biomarker for EBV-associated HLH and FHL type 2 and 3 (Toga *et al*, 2010; Wada *et al*, 2014). However, most biomarkers were identified retrospectively, requiring validation in prospective studies. Some biomarkers are not routinely assessed in HLH patients and may not be evaluated timely for rapid diagnostic purposes.

Treatment strategies in HLH

Conventional therapy

In parallel with the diagnostic criteria, therapeutic guidelines for HLH were published by the Histiocyte Society in 1994 and revised in 2004 (Henter *et al*, 2007). The HLH-2004 treatment protocol is based on chemo-immunotherapy, combining chemotherapeutic agents with immunosuppressive drugs to limit the proliferation and activation of immune cells and to halt the cytokine storm. It consists of systemic treatment with dexamethasone, etoposide and ciclosporin A, supplemented with intrathecal methotrexate and corticosteroids in cases with severe CNS involvement. Dexamethasone is the preferred steroid treatment, particularly in patients with CNS dysfunction, due to its better blood-brain barrier penetration. Prednisone, prednisolone and methylprednisolone are often administered in secondary or milder HLH cases. Additional supportive care is indicated to resolve bleeding problems and correct severe cytopenias. Anti-pathogen therapy is crucial to eliminate any triggering agents, reduce antigenaemia and temper on-going antigen presentation. Intravenous immunoglobulins can be of aid, particularly in infection-associated HLH. Liposomal amphotericin B is specifically indicated for HLH complicating visceral leishmaniasis. Rituximab, a B-cell targeting anti-CD20 antibody, can reduce viral load and improve EBV-associated HLH (Henter *et al*, 2007; Janka & Lehmberg, 2013, 2014).

Etoposide is a cornerstone in the HLH-2004 protocol. It exerts its therapeutic effects on three different levels. In murine FHL, etoposide acted directly and selectively on activated T cells to induce apoptosis, not affecting inactive naive or memory T cells (Johnson *et al*, 2014). Additionally, as a topoisomerase II inhibitor, etoposide induces DNA errors in fast dividing cells, provoking immunologically silent

apoptosis of activated immune cells and limiting the release of inflammatory alarmins due to necrosis or pyroptosis. Etoposide may thus partially substitute for perforin-triggered apoptosis (Palmblad *et al*, 2014). Thirdly, etoposide can inhibit EBV nuclear antigen synthesis and transformation of EBV-infected cells, of relevance in EBV-associated HLH (Rouphael *et al*, 2007).

Despite the improvements made to the HLH-94 protocol, an estimated 30% of HLH patients does not respond to conventional therapy. For refractory patients, a salvage treatment consisting of liposomal doxorubicin, etoposide and methylprednisolone, the so-called DEP regimen, recently showed encouraging results in the first prospective clinical trial for adult HLH (Wang *et al*, 2015). Plasma exchange, historically used to treat HLH in the 1980s, might still be of use as salvage therapy, because it controls hypercytokinaemia and treats bleeding tendencies (Janka & Lehmberg, 2014).

Ultimately, for primary HLH as well as severe refractory secondary HLH, allogeneic HSCT represents the final solution. Classifying the extent of the cytotoxic deficiency can give guidance as to which patients inevitably require HSCT for prolonged survival. If the cytotoxic function can be restored by *in vitro* stimulation, HSCT appears less urgent but may still be recommended (Horne *et al*, 2005). The use of myeloablative conditioning regimens, containing etoposide, busulfan and cyclophosphamide, was recommended in the HLH-2004 protocol (Henter *et al*, 2007), but has been shown to correlate with a relatively poor outcome of 50–70% survival. Starting the last decade, reduced-intensity conditioning regimens, consisting of alemtuzumab, fludarabine and melphalan/treosulfan, have significantly improved survival rates up to 90%. However, reduced-intensity regimens are associated with increased prevalence of mixed haematopoietic stem cell chimerism (Henter *et al*, 2007; Janka & Lehmberg, 2013, 2014). Nonetheless, this might not crucially influence patient survival as data in murine FHL indicate that mixed haematopoietic or CTL chimerism, with an engraftment of as little as 10–20% of perforin-expressing cells, is adequate to restore perforin-dependent immunoregulation and to prevent HLH development (Terrell & Jordan, 2013).

While allogeneic HSCT is currently the only permanent solution for primary HLH, in the future, autologous HSCT might be applicable, combined with gene therapy to correct the genetic defect. Transfer of a functional perforin gene (*Prf1*) into autologous haematopoietic stem cells from perforin-deficient mice restored perforin expression, partially repaired the cytotoxic defect, and attenuated HLH symptoms after viral challenge, provided that at least 30% engraftment was attained (Carmo *et al*, 2014). In a mouse model of XLP, gene transfer also restored SAP expression and normalized cytotoxic function (Rivat *et al*, 2013). Autologous HSCT has been successfully performed in secondary HLH patients, mostly in lymphoma-associated HLH (Ohga *et al*, 1997; Shimazaki *et al*, 2000). As positive effects of autologous HSCT were reported in sJIA (Wulffraat, 2003), it might also prove beneficial in sJIA-associated HLH.

Ironically, autologous and allogeneic HSCT have likewise been reported to induce HLH in transplanted patients, probably related to the immunosuppressive conditioning regimen and heightened infection risk. A recent cohort estimated the incidence of HLH post HSCT at 4%. Etoposide-containing conditioning regimens reduced the risk of HLH (Kobayashi *et al*, 2014).

Alternative and new treatment strategies

As not all patients respond to conventional therapy, the search for novel treatments continues. Recent years have seen a shift from overall immunosuppression towards more targeted approaches using biologicals. These agents aim to directly eliminate pathologically activated T cells or to prevent the detrimental effects of the cytokine storm by targeting individual cytokines or entire cytokine signalling pathways.

To halt aberrantly activated T cells, anti-thymocyte globulins can be administered, as well as alemtuzumab, an anti-CD52 antibody that predominantly targets mature lymphocytes, but also histiocytes (Jordan *et al*, 2011; Janka & Lehmberg, 2013). In addition, a few cases have been reported in which the anti-CD25 antibody daclizumab could successfully treat paediatric and adult HLH. In HLH, daclizumab is anticipated to normalize sCD25 levels and to deplete hyperactivated T cells that have upregulated CD25 expression (Olin *et al*, 2008).

The application of different cytokine-targeting biologicals in HLH is derived from experimental models of primary and secondary HLH that pinpointed several cytokines as crucial disease-propagating factors: predominantly IFNγ, but also TNFα, IL6 and IL18 (reviewed in Brisse *et al*, 2015). Inhibitors of TNFα (etanercept, infliximab, adalimumab), IL6 (tocilizumab) and IL1β (anakinra, rilonacept, canakinumab) have been particularly applied in secondary HLH, mostly associated with rheumatological conditions, and were found to be effective in several case reports (Kobayashi *et al*, 2011; Nigrovic *et al*, 2011; Kahn & Cron, 2013; Rajasekaran *et al*, 2014; Schulert & Grom, 2015). Recently, the first report on a phase II clinical trial presented promising results using a human monoclonal anti-IFNγ antibody in combination with dexamethasone as a second-line therapy for refractory primary HLH patients (Jordan *et al*, 2015). Treatment was well tolerated and nine out of 13 patients enrolled achieved a satisfactory response. Of note, as no known causative HLH mutations were found in four patients, it is possible that secondary HLH episodes may also benefit from this new therapy (Jordan *et al*, 2015). Nonetheless, it must be kept in mind that, as mentioned above, the cytokine profile of HLH patients can vary with underlying aetiologies and triggering factors, indicating that rational personalized approaches are necessary to obtain maximal efficacy of specific cytokine-targeting therapies.

Paradoxically, the use of biologicals has also been associated with the emergence of HLH in different autoimmune/autoinflammatory disorders and in haematological neoplasms (Brito-Zerón *et al*, 2016). Especially in rheumatologic disorders, anti-cytokine treatment has been linked to HLH development (Kobayashi *et al*, 2011; Schulert & Grom, 2015). Theoretically, biologicals have the capacity to destabilize immune homeostasis or create an imbalance in the cytokine network, thus favouring the occurrence of serious infections, in turn, leading to HLH. Recently, a comprehensive overview was published of adult patients who developed HLH following different biological therapies. Anti-TNF agents were the main inducers of HLH and the majority of episodes (67%) was co-triggered by a systemic infection (Brito-Zerón *et al*, 2016). A post-marketing surveillance study in sJIA patients on tocilizumab treatment revealed the occurrence of HLH in 5·8% of patients. Here, infection was a co-trigger in 29% of patients and active sJIA was considered the main contributing factor to HLH development (Yokota *et al*, 2015). Interestingly, tocilizumab might be temporarily linked to HLH onset in some patients, but it can also aide rapid resolution of the HLH episode in the same patients (Kobayashi *et al*, 2011). Similarly, the HLH syndrome occurring during anakinra treatment in some sJIA patients was improved by dose escalation of anakinra, indicating that biologicals can indeed constitute a triggering and therapeutic agent for the same disease (Nigrovic *et al*, 2011; Kahn & Cron, 2013). In a study assessing the impact of canakinumab treatment on HLH incidence, the occurrence of HLH was reported to remain equal between canakinumab-treated and placebo-treated sJIA patients, in fact, infections were deemed the predominant triggers of HLH cases (Grom *et al*, 2016). Thus, biologicals themselves do not appear to constitute the direct cause of HLH onset; rather, biological-induced susceptibility to infections might play a role. Of note, although the administration of cytokine-targeting drugs in underlying disorders is unable to prevent the emergence of complicating HLH, the HLH symptoms may be milder or partially masked, hindering diagnosis (Shimizu *et al*, 2012; Ravelli *et al*, 2016b).

In addition to targeting individual cytokines, which may be insufficient during severe hypercytokinaemia, cytokine signalling pathways can be targeted to avoid an imbalance in the cytokine network. Inhibition of Janus kinases, key transducers of multiple cytokine-mediated signals, via ruxolitinib may serve this purpose (Das *et al*, 2016). Peroxisome proliferator-activated receptor-γ agonists have also been projected as ideal candidates. They interfere with the activation of the NF-κB pathway and combine a broad anti-inflammatory mode of action with antiviral capacities (Chuang *et al*, 2007; Hsieh *et al*, 2010).

Other promising targets for future therapy are primarily based on research in HLH animal models and include the induction of T cell exhaustion through the stimulation of inhibitory receptors like programmed cell death 1 (PDCD1/PD-1), the administration of anti-inflammatory IL10 or IL18BP to restore cytokine balances, the application of TLR

Table IV. Potential treatment strategies in HLH.

Conventional therapy	Alternative treatments	Biological therapy
Corticosteroids	DEP regimen	Rituximab (anti-CD20)
Dexamethasone	Liposomal doxorubicin	T-cell targeting drugs
Methylprednisolone	Etoposide	Anti-thymocyte globulins
Prednisolone	Methylprednisolone	Alemtuzumab (anti-CD52)
Prednisone	Plasma exchange	Daclizumab (anti-CD25)
Etoposide	Allogenic HSCT	Cytokine-targeting drugs
Ciclosporin A	Reduced-intensity conditioning	Etanercept (TNFα blockade)
Methotrexate	Alemtuzumab	Infliximab (TNFα blockade)
Allogenic HSCT	Fludarabin	Adalimumab (TNFα blockade)
Myeloablative conditioning:	Melphalan	Tocilizumab (IL6 blockade)
Etoposide	Treosulfan	Anakinra (IL1 blockade)
Busulfan		Rilonacept (IL1 blockade)
Cyclophosphamide	**Future treatment strategies**	Canakinumab (IL1 blockade)
	Gene therapy and autologous HSCT	Anti-IFNγ monoclonal Ab
Supportive care	Ruxolitinib (JAK inhibitor)	
Anti-pathogen therapy	PPAR-γ agonists or NF-κB blockade	
Antivirals	Stimulation of inhibitory TCRs	
Antibiotics	Administration of IL10 or IL18BP	
Antimycotics	TLR blockade	
Liposomal amphotericin B	Targeting DCs or Ag presentation	
Intravenous immunoglobulins	Blocking alarmins (HMGB1, IL33, etc.)	

Ab, antibody; Ag, antigen; DCs, dendritic cells; HLH, haemophagocytic lymphohistiocytosis; HMGB1, high mobility group box 1; HSCT, haematopoietic stem cell transplantation; IFNγ, γ-interferon; IL, interleukin; IL18BP, IL18 binding protein; JAK, Janus kinases; NF, nuclear factor; PPAR, peroxisome proliferator-activated receptor; TCRs, T cell receptors; TLR, Toll-like receptor.

antagonists or blocking TLR signalling pathways to halt chronic TLR activation, targeting dendritic cells as the main drivers of on-going antigen stimulation or suppressing antigen presentation itself (reviewed in Brisse *et al*, 2015). Recently, neutralizing antibodies and antagonists targeting the alarmin HMGB1 were proposed for treating HLH, to reduce the immunostimulatory load of necrosis- and pyroptosis-derived danger signals. The efficacy of this strategy has already been demonstrated in other models of systemic sterile and infectious inflammation (Palmblad *et al*, 2014). Also in this category, blocking the alarmin IL33, via its receptor ST2/IL1RL1, may constitute a novel therapeutic approach (Rood *et al*, 2016). The treatment strategies discussed above are summarized in Table IV.

Conclusion

This review covers the spectrum of HLH symptoms, highlighting possible distinguishing features between primary and secondary HLH. Recent advances in specific diagnostic markers and protocols for different HLH subtypes will increase future recognition of the syndrome and allow for more rapid and accurate diagnosis. Combined with the increased application of targeted therapies, patient prognosis will probably improve. Nevertheless, personalizing therapy remains of utmost importance in future research efforts to maximize the efficacy of existing drugs and tailor novel drugs to individual patient needs.

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Author contributions

E.B. wrote the review article. P.M. and C.H.W. thoroughly and critically revised the manuscript.

References

Akashi, K., Hayashi, S., Gondo, H., Mizuno, S., Harada, M., Tamura, K., Yamasaki, K., Shibuya, T., Uike, N., Okamura, T., Miyamoto, T. & Niho, Y. (1994) Involvement of interferon-γ and macrophage colony-stimulating factor in pathogenesis of haemophagocytic lymphohistiocytosis in adults. *British Journal of Haematology*, **87**, 243–250.

Aricò, M., Danesino, C., Pende, D. & Moretta, L. (2001) Pathogenesis of haemophagocytic lymphohistiocytosis. *British Journal of Haematology*, **114**, 761–769.

Avcin, T., Tse, S.M.L., Schneider, R., Ngan, B. & Silverman, E.D. (2006) Macrophage activation syndrome as the presenting manifestation of rheumatic diseases in childhood. *Journal of Pediatrics*, **148**, 683–686.

Bay, A., Coskun, E., Oztuzcu, S., Ergun, S., Yilmaz, F. & Aktekin, E. (2013) Evaluation of the plasma micro RNA expression levels in secondary hemophagocytic lymphohistiocytosis. *Mediterranean Journal of Hematology and Infectious Diseases*, **5**, e2013066.

Beer, T. & Vadakara, J. (2015) Etiologies and short-term mortality in patients with ultraelevated serum ferritin. *Southern Medical Journal*, **108**, 574–578.

Bode, S.F.N., Ammann, S., Al-Herz, W., Bataneant, M., Dvorak, C.C., Gehring, S., Gennery, A., Gilmour, K.C., Gonzalez-Granado, L.I., GroB-Wieltsch, U., Ifversen, M., Lingman-Framme, J., Matthes-Martin, S., Mesters, R., Meyts, I., vanMontfrans, J.M., Pachlopnik Schmid, J., Pai, S.-Y., Soler-Palacin, P., Schuermann, U., Schuster, V., Seidel, M.G., Speckmann, C., Stepensky, P., Sykora, K.W., Tesi, B., Vraetz, T., Waruiru, C., Bryceson, Y.T., Moshous, D., Lehmberg, K., Jordan, M.B. & Ehl, S.; on behalf of the Inborn Errors Working Party of the EBMT. (2015) The syndrome of hemophagocytic lymphohistiocytosis in primary immunodeficiencies: implications for differential diagnosis and pathogenesis. *Haematologica*, **100**, 978–988.

Bracaglia, C., Marafon, D., Caiello, I., de Graaf, K., Guilhot, F., Ferlin, W., Davì, S., Schulert, G., Ravelli, A., Grom, A., Nelson, R., de Min, C. & De Benedetti, F. (2015) High levels of interferon-gamma (IFNγ) in macrophage activation syndrome (MAS) and CXCL9 levels as a biomarker for IFNγ production in MAS. *Pediatric Rheumatology*, **13**, O84.

Brisse, E., Wouters, C.H. & Matthys, P. (2015) Hemophagocytic lymphohistiocytosis (HLH): a heterogeneous spectrum of cytokine-driven immune disorders. *Cytokine & Growth Factor Reviews*, **26**, 263–280.

Brito-Zerón, P., Bosch, X., Pérez-de-Lis, M., Pérez-Álvarez, R., Fraile, G., Gheitasi, H., Retamozo, S., Bové, A., Monclús, E., Escoda, O., Moreno, A., López-Guillermo, A., Khamashta, M.A. & Ramos-Casals, M. (2016) Infection is the major trigger of hemophagocytic syndrome in adult patients treated with biological therapies. *Seminars in Arthritis and Rheumatism*, **45**, 391–399.

Bryceson, Y.T., Pende, D., Maul-Pavicic, A., Gilmour, K.C., Ufheil, H., Vraetz, T., Chiang, S.C., Marcenaro, S., Meazza, R., Bondzio, I., Walshe, D., Janka, G., Lehmberg, K., Beutel, K., Stadt, U.Zur, Binder, N., Arico, M., Moretta, L., Henter, J.I. & Ehl, S. (2012) A prospective evaluation of degranulation assays in the rapid diagnosis of familial hemophagocytic syndromes. *Blood*, **119**, 2754–2763.

Canna, S.W. & Behrens, E.M. (2012) Not all hemophagocytes are created equally : appreciating the heterogeneity of the hemophagocytic syndromes. *Current Opinion in Rheumatology*, **24**, 113–118.

Canna, S.W., Costa-reis, P., Bernal, W.E., Chu, N., Sullivan, K., Paessler, M. & Behrens, E.M. (2014) Alternative activation of laser-captured murine hemophagocytes. *Arthritis & Rheumatism*, **66**, 1666–1671.

Carmo, M., Risma, K.A., Arumugam, P., Tiwari, S., Hontz, A.E., Montiel-Equihua, C.A., Alonso-Ferrero, M.E., Blundell, M.P., Schambach, A., Baum, C., Malik, P., Thrasher, A.J., Jordan, M.B. & Gaspar, H.B. (2014) Perforin gene transfer into hematopoietic stem cells improves immune dysregulation in murine models of perforin deficiency. *Molecular Therapy*, **23**, 737–745.

Castillo, L. & Carcillo, J. (2009) Secondary hemophagocytic lymphohistiocytosis and severe sepsis/systemic inflammatory response syndrome/multiorgan dysfunction syndrome/macrophage activation syndrome share common intermediate phenotypes on a spectrum of inflammation. *Pediatric Critical Care Medicine*, **10**, 387–92.

Cetica, V., Sieni, E., Pende, D., Danesino, C., De Fusco, C., Locatelli, F., Micalizzi, C., Putti, M.C., Biondi, A., Fagioli, F., Moretta, L., Griffiths, G.M., Luzzatto, L. & Aricò, M. (2016) Genetic predisposition to hemophagocytic lymphohistiocytosis: report on 500 patients from the Italian registry. *The Journal of Allergy and Clinical Immunology*, **137**, 188–196.

Chen, J., Wang, X., He, P., Li, Y., Si, M., Fan, Z., Chang, X., Xie, Q. & Jiao, X. (2016) Viral etiology, clinical and laboratory features of adult

hemophagocytic lymphohistiocytosis. *Journal of Medical Virology*, **88**, 541–549.

Chiossone, L., Audonnet, S., Chetaille, B., Chasson, L., Farnarier, C., Berda-Haddad, Y., Jordan, S., Koszinowski, U.H., Dalod, M., Mazodier, K., Novick, D., Dinarello, C.A., Vivier, E. & Kaplanski, G. (2012) Protection from inflammatory organ damage in a murine model of hemophagocytic lymphohistiocytosis using treatment with IL-18 binding protein. *Frontiers in Immunology*, **3**, 239.

Chuang, H.-C., Lay, J.-D., Hsieh, W.-C. & Su, I.-J. (2007) Pathogenesis and mechanism of disease progression from hemophagocytic lymphohistiocytosis to Epstein-Barr virus-associated T-cell lymphoma: nuclear factor-kappa B pathway as a potential therapeutic target. *Cancer Science*, **98**, 1281–7.

Cifaldi, L., Prencipe, G., Caiello, I., Bracaglia, C., Locatelli, F., De Benedetti, F. & Strippoli, R. (2015) Inhibition of natural killer cell cytotoxicity by interleukin-6: implications for the pathogenesis of macrophage activation syndrome. *Arthritis & Rheumatology*, **67**, 3037–46.

Créput, C., Galicier, L., Buyse, S. & Azoulay, E. (2008) Understanding organ dysfunction in hemophagocytic lymphohistiocytosis. *Intensive Care Medicine*, **34**, 1177–87.

Das, R., Guan, P., Sprague, L., Verbist, K., Tedrick, P., An, Q.A., Cheng, C., Kurachi, M., Levine, R., Wherry, J., Canna, S.W., Behrens, E.M. & Nichols, K.E. (2016) Janus kinase inhibition lessens inflammation and ameliorates disease in murine models of hemophagocytic lymphohistiocytosis. *Blood*, **127**, 1666–1675.

Davì, S., Consolaro, A., Guseinova, D., Pistorio, A., Ruperto, N., Martini, A., Cron, R.Q. & Ravelli, A. (2011) An international consensus survey of diagnostic criteria for macrophage activation syndrome in systemic juvenile idiopathic arthritis. *The Journal of Rheumatology*, **38**, 764–8.

Davì, S., Minoia, F., Pistorio, A., Horne, A., Consolaro, A., Rosina, S., Bovis, F., Cimaz, R., Gamir, M.L., Ilowite, N.T., Kone-Paut, I., Feitosa de Oliveira, S.K., McCurdy, D., Silva, C.A., Sztajnbok, F., Tsitsami, E., Unsal, E., Weiss, J.E., Wulffraat, N., Abinun, M., Aggarwal, A., Apaz, M.T., Astigarraga, I., Corona, F., Cuttica, R., D'Angelo, G., Eisenstein, E.M., Hashad, S., Lepore, L., Mulaosmanovic, V., Nielsen, S., Prahalad, S., Rigante, D., Stanevicha, V., Sterba, G., Susic, G., Takei, S., Trauzeddel, R., Zletni, M., Ruperto, N., Martini, A., Cron, R.Q. & Ravelli, A. (2014) Performance of current guidelines for diagnosis of macrophage activation syndrome complicating systemic juvenile idiopathic arthritis. *Arthritis & Rheumatology*, **66**, 2871–80.

Emmenegger, U., Zehnder, R., Frey, U., Reimers, A., Spaeth, P.J. & Neftel, K.A. (2000) Elevation of soluble Fas and soluble Fas ligand in reactive macrophage activation syndromes. *American Journal of Hematology*, **64**, 116–119.

Fardet, L., Galicier, L. & Lambotte, O. (2014) Development and validation of a score for the diagnosis of reactive hemophagocytic syndrome (HScore). *Arthritis & Rheumatism*, **66**, 2613–20.

Fisman, D.N. (2000) Hemophagocytic syndromes and infection. *Emerging Infectious Diseases*, **6**, 601–8.

Gorelik, M., Fall, N., Altaye, M., Barnes, M.G., Thompson, S.D., Grom, A.A. &Hirsch, R. (2013) Follistatin-like protein 1 and the ferritin/erythrocyte sedimentation rate ratio are potential biomarkers for dysregulated gene expression and macrophage activation syndrome in systemic juvenile idiopathic arthritis. *The Journal of Rheumatology*, **40**, 1191–9.

Grom, A.A. (2004) Natural killer cell dysfunction: a common pathway in systemic-onset juvenile rheumatoid arthritis, macrophage activation syndrome, and hemophagocytic lymphohistiocytosis? *Arthritis and Rheumatism*, **50**, 689–98.

Grom, A.A., Ilowite, N.T., Pascual, V., Brunner, H.I., Martini, A., Lovell, D., Ruperto, N., Leon, K., Lheritier, K. & Abrams, K. (2016) Canakinumab in systemic juvenile idiopathic arthritis: impact on the rate and clinical presentation of macrophage activation syndrome. *Arthritis & Rheumatology*, **68**, 218–228.

Henter, J.I., Horne, A., Arico, M., Egeler, R.M., Webb, D., Winiarski, J. & Janka, G. (2007) HLH-2004 : diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pedriatric Blood & Cancer*, **12**, 4–131.

Hibi, S., Ikushima, S., Fujiwara, F., Hashida, T., Tsunamoto, K., Todo, S. & Imashuku, S. (1995) Serum and urine beta-2-microglobulin in hemophagocytic syndrome. *Cancer*, **75**, 1700–5.

Holzinger, D., Fall, N., Grom, A., de Jager, W., Vastert, S., Strippoli, R., Bracaglia, C., Sundberg, E., Horne, A., Ehl, S., De Benedetti, F., Beutel, K. & Foell, D. (2015) S100A12 as diagnostic tool in the differential diagnosis of sJIA associated MAS vs. hereditary or acquired HLH. *Pediatric Rheumatology*, **13**, O64.

Horne, A., Janka, G., Egeler, R.M., Gadner, H., Imashuku, S., Ladisch, S., Locatelli, F., Montgomery, S.M., Webb, D., Winiarski, J., Filipovich, A.H. & Henter, J.I. (2005) Haematopoietic stem cell transplantation in haemophagocytic lymphohistiocytosis. *British Journal of Haematology*, **129**, 622–630.

Horne, A., Trottestam, H., Aricò, M., Egeler, R.M., Filipovich, A.H., Gadner, H., Imashuku, S., Ladisch, S., Webb, D., Janka, G. & Henter, J.I. (2008) Frequency and spectrum of central nervous system involvement in 193 children with haemophagocytic lymphohistiocytosis. *British Journal of Haematology*, **140**, 327–335.

Hsieh, W.-C., Lan, B.-S., Chen, Y.-L., Chang, Y., Chuang, H.-C. & Su, I.-J. (2010) Efficacy of peroxisome proliferator activated receptor agonist in the treatment of virus-associated haemophagocytic syndrome in a rabbit model. *Antiviral Therapy*, **15**, 71–81.

Ibarra, M.F., Klein-Gitelman, M., Morgan, E., Proytcheva, M., Sullivan, C., Morgan, G., Pachman, L.M. & O'Gorman, M.R.G. (2011) Serum neopterin levels as a diagnostic marker of hemophagocytic lymphohistiocytosis syndrome. *Clinical and Vaccine Immunology*, **18**, 609–14.

Ishii, E., Ohga, S., Imashuku, S., Yasukawa, M., Tsuda, H., Miura, I., Yamamoto, K., Horiuchi, H., Takada, K., Ohshima, K., Nakamura, S., Kinukawa, N., Oshimi, K. & Kawa, K. (2007) Nationwide survey of hemophagocytic lymphohistiocytosis in Japan. *International Journal of Hematology*, **86**, 58–65.

Janka, G.E. (2007) Familial and acquired hemophagocytic lymphohistiocytosis. *European Journal of Pediatrics*, **166**, 95–109.

Janka, G.E. & Lehmberg, K. (2013) Hemophagocytic lymphohistiocytosis: pathogenesis and treatment. *Hematology/the Education Program of the American Society of Hematology*, **2013**, 605–611.

Janka, G.E. & Lehmberg, K. (2014) Hemophagocytic syndromes - an update. *Blood Reviews*, **28**, 135–142.

Jenkins, R.W., Clarke, C.J., Lucas, J.T., Shabbir, M., Wu, B.X., Simbari, F., Mueller, J., Hannun, Y.A., Lazarchick, J. &Shirai, K. (2013) Evaluation of the role of secretory sphingomyelinase and bioactive sphingolipids as biomarkers in hemophagocytic lymphohistiocytosis. *American Journal of Hematology*, **88**, E265–72.

Johnson, T.S., Terrell, C.E., Millen, S.H., Katz, J.D., Hildeman, D.A. & Jordan, M.B. (2014) Etoposide selectively ablates activated T cells to control the immunoregulatory disorder hemophagocytic lymphohistiocytosis. *The Journal of Immunology*, **192**, 84–91.

Jordan, M.B., Allen, C.E., Weitzman, S., Filipovich, A.H. & McClain, K.L. (2011) How I treat hemophagocytic lymphohistiocytosis. *Blood*, **118**, 4041–52.

Jordan, M., Locatelli, F., Allen, C., De Benedetti, F., Grom, A.A., Ballabio, M., Ferlin, W.G., NI-0501-04 Study Group & De Min, C. (2015) A novel targeted approach to the treatment of hemophagocytic lymphohistiocytosis (HLH) with an anti-interferon gamma (IFNγ) monoclonal antibody (mAb), NI-0501: first results from a pilot phase 2 study in children with primary HLH. *Blood*, **126**, LBA–3.

Kahn, P.J. & Cron, R.Q. (2013) Higher-dose anakinra is effective in a case of medically refractory macrophage activation syndrome the journal of rheumatology is a monthly international serial edited by Earl D. Silverman featuring research articles on clinic. *The Journal of Rheumatology*, **40**, 5–7.

Kobayashi, M., Takahashi, Y., Yamashita, H., Kaneko, H. & Mimori, A. (2011) Benefit and a possible risk of tocilizumab therapy for adult-onset Still's disease accompanied by macrophage-activation syndrome. *Modern Rheumatology/the Japan Rheumatism Association*, **21**, 92–6.

Kobayashi, R., Tanaka, J., Hashino, S., Ota, S., Torimoto, Y., Kakinoki, Y., Yamamoto, S., Kurosawa, M., Hatakeyama, N., Haseyama, Y., Sakai, H., Sato, K. & Fukuhara, T. (2014) Etoposide-containing conditioning regimen reduces the occurrence of hemophagocytic

lymphohistiocytosis after SCT. *Bone Marrow Transplantation*, **49**, 254–7.

Koorts, A.M. & Viljoen, M. (2011) Acute phase proteins: ferritin and ferritin isoforms. In: Acute Phase Proteins - Regulation and Functions of Acute Phase Proteins (ed. by F. Veas), pp. 153–184. InTech: Rijeka, Croatia.

Kostik, M.M., Dubko, M.F., Masalova, V.V., Snegireva, L.S., Kornishina, T.L., Chikova, I.A., Likhacheva, T.S., Isupova, E.A., Glebova, N.I., Kuchinskaya, E.M., Balbotkina, E.V., Buchinskaya, N.V., Kalashnikova, O.V. & Chasnyk, V.G. (2015) Identification of the best cutoff points and clinical signs specific for early recognition of macrophage activation syndrome in active systemic juvenile idiopathic arthritis. *Seminars in Arthritis and Rheumatism*, **44**, 417–22.

Kuriyama, T., Takenaka, K., Kohno, K., Yamauchi, T., Daitoku, S., Yoshimoto, G., Kikushige, Y., Kishimoto, J., Abe, Y., Harada, N., Miyamoto, T., Iwasaki, H., Teshima, T. & Akashi, K. (2012) Engulfment of hematopoietic stem cells caused by down-regulation of CD47 is critical in the pathogenesis of hemophagocytic lymphohistiocytosis. *Blood*, **120**, 4058–67.

Lehmberg, K., Pink, I., Eulenburg, C., Beutel, K., Maul-Pavicic, A. & Janka, G. (2013) Differentiating macrophage activation syndrome in systemic juvenile idiopathic arthritis from other forms of hemophagocytic lymphohistiocytosis. *The Journal of Pediatrics*, **162**, 1245–51.

Lehmberg, K., Mcclain, K.L., Janka, G.E. & Allen, C.E. (2014) Determination of an appropriate cut-off value for ferritin in the diagnosis of hemophagocytic lymphohistiocytosis. *Pediatric Blood & Cancer*, **61**, 2101–3.

Lehmberg, K., Nichols, K.E., Henter, J.-I., Girschikofsky, M., Greenwood, T., Jordan, M., Kumar, A., Minkov, M., La Rosée, P. & Weitzman, S.; Study Group on Hemophagocytic Lymphohistiocytosis Subtypes of the Histiocyte Society (2015) Consensus recommendations for the diagnosis and management of hemophagocytic lymphohistiocytosis associated with malignancies. *Haematologica*, **100**, 997–1004.

Maruoka, H., Inoue, D., Takiuchi, Y., Nagano, S., Arima, H., Tabata, S., Matsushita, A., Ishikawa, T., Oita, T. & Takahashi, T. (2014) IP-10/CXCL10 and MIG/CXCL9 as novel markers for the diagnosis of lymphoma-associated hemophagocytic syndrome. *Annals of Hematology*, **93**, 393–401.

Maruyama, J. & Inokuma, S. (2010) Cytokine profiles of macrophage activation syndrome associated with rheumatic diseases. *The Journal of Rheumatology*, **37**, 967–73.

Mazodier, K., Marin, V., Novick, D., Farnarier, C., Robitail, S., Schleinitz, N., Veit, V., Paul, P., Rubinstein, M., Dinarello, C.A., Harlé, J.-R. & Kaplanski, G. (2005) Severe imbalance of IL-18/IL-18BP in patients with secondary hemophagocytic syndrome. *Blood*, **106**, 3483–9.

McCoy, M.W., Moreland, S.M. & Detweiler, C.S. (2012) Hemophagocytic macrophages in murine typhoid fever have an anti-inflammatory phenotype. *Infection and Immunity*, **80**, 3642–9.

Meeths, M., Horne, A., Sabel, M., Bryceson, Y.T. & Henter, J.-I. (2015) Incidence and clinical presentation of primary hemophagocytic lymphohistiocytosis in Sweden. *Pediatric Blood & Cancer*, **62**, 346–352.

Niece, J.A., Roger, Z.R., Ahmad, N., Langevin, A.-M. & McClain, K.L. (2010) Hemophagocytic lymphohistiocytosis in Texas: observations on ethnicity and race. *Pediatric Blood & Cancer*, **54**, 424–428.

Nigrovic, P.A., Mannion, M., Prince, F.H.M., Zeft, A., Rabinovich, C.E., van Rossum, M.A.J., Cortis, E., Pardeo, M., Miettunen, P.M., Janow, G., Birmingham, J., Eggebeen, A., Janssen, E., Shulman, A.I., Son, M.B., Hong, S., Jones, K., Ilowite, N.T., Cron, R.Q. & Higgins, G.C. (2011) Anakinra as first-line disease-modifying therapy in systemic juvenile idiopathic arthritis: report of forty-six patients from an international multicenter series. *Arthritis and Rheumatism*, **63**, 545–55.

Nold-Petry, C.A., Lehrnbecher, T., Jarisch, A., Schwabe, D., Pfeilschifter, J.M., Muhl, H. & Nold, M.F. (2010) Failure of interferon gamma to induce the anti-inflammatory interleukin 18 binding protein in familial hemophagocytosis. *PLoS ONE*, **5**, e8663.

Ohga, S., Nomura, A., Kai, T., Matsuzaki, A., Inaba, S., Suda, M. & Ueda, K. (1997) Case report prolonged resolution of hemophagocytic lymphohistiocytosis following myeloablative chemotherapy and subsequent autologous peripheral blood stem cell transplantation. *Bone Marrow Transplantation*, **19**, 633–635.

Olin, R.L., Nichols, K.E., Naghashpour, M., Wasik, M., Shelly, B., Stadtmauer, E.A. & Vogl, D.T. (2008) Successful use of the anti-CD25 antibody daclizumab in an adult patient with hemophagocytic lymphohistiocytosis. *American Journal of Hematology*, **83**, 747–749.

Osugi, Y., Hara, J., Tagawa, S., Takai, K., Hosoi, G., Matsuda, Y., Ohta, H., Fujisaki, H., Kobayashi, M., Sakata, N., Kawa-Ha, K., Okada, S. & Tawa, A. (1997) Cytokine production regulating Th1 and Th2 cytokines in hemophagocytic lymphohistiocytosis. *Blood*, **89**, 4100–3.

Ozen, S., Dai, A., Coskun, E., Oztuzcu, S., Ergun, S., Aktekin, E., Yavuz, S. & Bay, A. (2014) Importance of hyperbilurubinemia in differentiation of primary and secondary hemophagocytic lymphohistiocytosis in pediatric cases. *Mediterranean Journal of Hematology and Infectious Diseases*, **6**, e2014067.

Palmblad, K., Schierbeck, H., Sundberg, E., Horne, A.-C., Harris, H.E., Henter, J.-I., Antoine, D.J. & Andersson, U. (2014) High systemic levels of the cytokine-inducing HMGB1 isoform secreted in severe macrophage activation syndrome. *Molecular Medicine*, **20**, 538–47.

Parodi, A., Davì, S., Pringe, A.B., Pistorio, A., Ruperto, N., Magni-Manzoni, S., Miettunen, P., Bader-Meunier, B., Espada, G., Sterba, G., Ozen, S., Wright, D., Magalhães, C.S., Khubchandani, R., Michels, H., Woo, P., Iglesias, A., Guseinova, D., Bracaglia, C., Hayward, K., Wouters, C., Grom, A., Vivarelli, M., Fischer, A., Breda, L., Martini, A. & Ravelli, A.; on behalf of the Lupus Working Group of the Paediatric Rheumatology European Society. (2009) Macrophage activation syndrome in juvenile systemic lupus erythematosus: a multinational multicenter study of thirty-eight patients. *Arthritis and Rheumatism*, **60**, 3388–99.

Put, K., Avau, A., Brisse, E., Mitera, T., Put, S., Proost, P., Bader-Meunier, B., Westhovens, R., Van den Eynde, B.J., Orabona, C., Fallarino, F., De Somer, L., Tousseyn, T., Quartier, P., Wouters, C. & Matthys, P. (2015) Cytokines in systemic juvenile idiopathic arthritis and haemophagocytic lymphohistiocytosis: tipping the balance between interleukin-18 and interferon-γ. *Rheumatology*, **54**, 1507–1517.

Rajasekaran, S., Kruse, K., Kovey, K., Davis, A.T., Hassan, N.E., Ndika, A.N., Zuiderveen, S. & Birmingham, J. (2014) Therapeutic role of anakinra, an interleukin-1 receptor antagonist, in the management of secondary hemophagocytic lymphohistiocytosis/sepsis/multiple organ dysfunction/macrophage activating syndrome in critically ill children. *Pediatric Critical Care Medicine*, **15**, 401–8.

Ramos-Casals, M., Brito-Zerón, P., López-Guillermo, A., Khamashta, M.A. & Bosch, X. (2014) Adult haemophagocytic syndrome. *Lancet*, **383**, 1503–16.

Ravelli, A., Magni-Manzoni, S., Pistorio, A., Besana, C., Foti, T., Ruperto, N., Viola, S. & Martini, A. (2005) Preliminary diagnostic guidelines for macrophage activation syndrome complications systemic juvenile idiopathic arthritis. *Journal of Pediatrics*, **146**, 598–604.

Ravelli, A., Minoia, F., Davì, S., Horne, A., Bovis, F., Pistorio, A., Aricò, M., Avcin, T., Behrens, E.M., De Benedetti, F., Filipovic, L., Grom, A.A., Henter, J.-I., Ilowite, N.T., Jordan, M.B., Khubchandani, R., Kitoh, T., Lehmberg, K., Lovell, D.J., Miettunen, P., Nichols, K.E., Ozen, S., Pachlopnik Schmid, J., Ramanan, A.V., Russo, R., Schneider, R., Sterba, G., Uziel, Y., Wallace, C., Wouters, C., Wulffraat, N., Demirkaya, E., Brunner, H.I., Martini, A., Ruperto, N. & Cron, R.Q. (2016a) 2016 classification criteria for macrophage activation syndrome complicating systemic juvenile idiopathic arthritis. *Annals of the Rheumatic Diseases*, **75**, 481–489.

Ravelli, A., Minoia, F., Davì, S., Horne, A., Bovis, F., Pistorio, A., Aricò, M., Avcin, T., Behrens, E.M., De Benedetti, F., Filipovic, A., Grom, A.A., Henter, J.-I., Ilowite, N.T., Jordan, M.B., Khubchandani, R., Kitoh, T., Lehmberg, K., Lovell, D.J., Miettunen, P., Nichols, K.E., Ozen, S., Pachlopnik Schmid, J., Ramanan, A.V., Russo, R., Schneider, R., Sterba, G., Uziel, Y., Wallace, C., Wouters, C., Wulffraat, N., Demirkaya, E., Brunner, H., Martini, A., Ruperto, N. & Cron, R.Q. (2016b) Expert consensus on dynamics of laboratory tests for diagnosis of macrophage activation syndrome complicating

systemic juvenile idiopathic arthritis. *RMD Open*, **2**, e000161.

Reinhardt, R.L., Liang, H.-E., Bao, K., Price, A.E., Mohrs, M., Kelly, B.L. & Locksley, R.M. (2015) A novel model for IFN-gamma-mediated autoinflammatory syndromes. *The Journal of Immunology*, **194**, 2358–2368.

Rivat, C., Booth, C., Alonso-Ferrero, M., Blundell, M., Sebire, N.J., Thrasher, A.J. & Gaspar, H.B. (2013) SAP gene transfer restores cellular and humoral immune function in a murine model of X-linked lymphoproliferative disease. *Blood*, **121**, 1073–6.

Rood, J.E., Rao, S., Paessler, M., Kreiger, P.A., Chu, N., Stelekati, E., Wherry, E.J. & Behrens, E.M. (2016) ST2 contributes to T cell hyperactivation and fatal hemophagocytic lymphohistiocytosis in mice. *Blood*, **127**, 426–435.

Rouphael, N.G., Talati, N.J., Vaughan, C., Cunningham, K., Moreira, R. & Gould, C. (2007) Infections associated with haemophagocytic syndrome. *The Lancet. Infectious Diseases.*, **7**, 814–822.

Ruscitti, P., Cipriani, P., Di Benedetto, P., Ciccia, F., Liakouli, V., Carubbi, F., Berardicurti, O., Rizzo, A., Triolo, G. & Giacomelli, R. (2015) Increased level of H-ferritin and its imbalance with L-ferritin, in bone marrow and liver of patients with adult onset Still's disease, developing macrophage activation syndrome, correlate with the severity of the disease. *Autoimmunity Reviews*, **14**, 429–437.

Saeed, H., Woods, R.R., Lester, J., Herzig, R., Gul, Z. & Monohan, G. (2015) Evaluating the optimal serum ferritin level to identify hemophagocytic lymphohistiocytosis in the critical care setting. *International Journal of Hematology*, **102**, 195–199.

Schaer, D.J., Schleiffenbaum, B., Kurrer, M., Imhof, A., Bächli, E., Fehr, J., Moller, H.J., Moestrup, S.K. & Schaffner, A. (2005) Soluble hemoglobin-haptoglobin scavenger receptor CD163 as a lineage-specific marker in the reactive hemophagocytic syndrome. *European Journal of Haematology*, **74**, 6–10.

Schulert, G.S. & Grom, A.A. (2015) Pathogenesis of macrophage activation syndrome and potential for cytokine- directed therapies. *Annual Review of Medicine*, **66**, 145–159.

Scott, R. & Robb-Smith, A. (1939) Histiocytic medullary reticulocytosis. *Lancet*, **2**, 194–198.

Shimazaki, C., Inaba, T. & Nakagawa, M. (2000) B-cell lymphoma-associated hemophagocytic syndrome. *Leukemia & Lymphoma*, **38**, 121–30.

Shimizu, M., Yokoyama, T., Yamada, K., Kaneda, H., Wada, H., Wada, T., Toma, T., Ohta, K., Kasahara, Y. & Yachie, A. (2010) Distinct cytokine profiles of systemic-onset juvenile idiopathic arthritis-associated macrophage activation syndrome with particular emphasis on the role of interleukin-18 in its pathogenesis. *Rheumatology*, **49**, 1645–53.

Shimizu, M., Nakagishi, Y., Kasai, K., Yamasaki, Y., Miyoshi, M., Takei, S. & Yachie, A. (2012) Tocilizumab masks the clinical symptoms of systemic juvenile idiopathic arthritis-associated macrophage activation syndrome: the diagnostic significance of interleukin-18 and interleukin-6. *Cytokine*, **58**, 287–94.

Shimizu, M., Nakagishi, Y., Inoue, N., Mizuta, M., Ko, G., Saikawa, Y., Kubota, T., Yamasaki, Y., Takei, S. & Yachie, A. (2015) Interleukin-18 for predicting the development of macrophage activation syndrome in systemic juvenile idiopathic arthritis. *Clinical Immunology*, **160**, 277–281.

Takahashi, N., Chubati, A., Miura, I., Nakamura, S. & Miura, T. (1999) Lymphoma-associated hemophagocytic syndrome in Japan. *Japanese Journal of Clinical Hematology*, **40**, 542–549 [in Japanese, abstract in English].

Tamura, K., Kanazawa, T., Tsukada, S., Kobayashi, T., Kawamura, M. & Morikawa, A. (2008) Increased serum monocyte chemoattractant protein-1, macrophage inflammatory protein-1 beta, and interleukin-8 concentration in hemophagocytic lymphohistiocytosis. *Pediatric Blood & Cancer*, **51**, 662–668.

Terrell, C.E. & Jordan, M.B. (2013) Mixed hematopoietic or T cell chimerism above a minimal threshold restores perforin-dependent immune regulation in perforin-deficient mice. *Blood*, **122**, 2618–21.

Teruya-Feldstein, J., Setsuda, J., Yao, X., Kingma, D.W., Straus, S., Tosato, G. & Jaffe, E.S. (1999) MIP-1alpha expression in tissues from patients with hemophagocytic syndrome. *Laboratory Investigation*, **79**, 1583–90.

Toga, A., Wada, T., Sakakibara, Y., Mase, S., Araki, R., Tone, Y., Toma, T., Kurokawa, T., Yanagisawa, R., Tamura, K., Nishida, N., Taneichi, H., Kanegane, H. & Yachie, A. (2010) Clinical Significance of Cloned Expansion and CD5 Down-Regulation in Epstein-Barr Virus (EBV)–Infected CD8 + T Lymphocytes in EBV-Associated Hemophagocytic Lymphohistiocytosis. *The Journal of Infectious Diseases*, **201**, 1923–1932.

Valade, S., Azoulay, E., Galicier, L., Boutboul, D., Zafrani, L., Stepanian, A., Canet, E., Lemiale, V., Venot, M., Veyradier, A. & Mariotte, E. (2015) Coagulation Disorders and Bleedings in Critically Ill Patients With Hemophagocytic Lymphohistiocytosis. *Medicine*, **94**, e1692.

Wada, T., Muraoka, M., Yokoyama, T., Toma, T., Kanegane, H. & Yachie, A. (2013) Cytokine Profiles in Children With Primary Epstein – Barr Virus Infection. *Pediatric Blood & Cancer*, **60**, E46–E48.

Wada, T., Yasumi, T., Toma, T., Hori, M., Maeda, S., Umeda, K., Heike, T., Adachi, S., Usami, I. & Yachie, A. (2014) Munc13-4 deficiency with CD5 downregulation on activated CD8 + T cells. *Pediatrics International*, **56**, 605–8.

Wang, Z., Wang, Y., Wang, J., Feng, C., Tian, L. & Wu, L. (2009) Early diagnostic value of low percentage of glycosylated ferritin in secondary hemophagocytic lymphohistiocytosis. *International Journal of Hematology*, **90**, 501–5.

Wang, Y., Huang, W., Hu, L., Cen, X., Li, L., Wang, J., Shen, J., Wei, N. & Wang, Z. (2015) Multicenter study of combination DEP regimen as a salvage therapy for adult refractory hemophagocytic lymphohistiocytosis. *Blood*, **126**, 2186–2193.

Weaver, L.K. & Behrens, E.M. (2014) Hyperinflammation, rather than hemophagocytosis, is the common link between macrophage activation syndrome and hemophagocytic lymphohistiocytosis. *Current Opinion in Rheumatology*, **26**, 562–9.

Wulffraat, N.M. (2003) Reduced perforin expression in systemic juvenile idiopathic arthritis is restored by autologous stem-cell transplantation. *Rheumatology*, **42**, 375–379.

Yasumi, T., Hori, M., Hiejima, E., Shibata, H., Izawa, K., Oda, H., Yoshioka, K., Nakagawa, K., Kawai, T., Nishikomori, R., Ohara, O. & Heike, T. (2015) Laboratory parameters identify familial haemophagocytic lymphohistiocytosis from other forms of paediatric haemophagocytosis. *British Journal of Haematology*, **170**, 532–538.

Yokota, S., Itoh, Y., Morio, T., Origasa, H., Sumitomo, N., Tomobe, M., Tanaka, K. & Minota, S. (2015) Tocilizumab in systemic juvenile idiopathic arthritis in a real-world clinical setting : results from 1 year of postmarketing surveillance follow-up of 417 patients in Japan. *Annals of Rheumatic Disease*, doi:10.1136/annrheumdis-2015-207818 [Epub ahead of print].

Zhang, K., Jordan, M.B., Marsh, R.A., Johnson, J.A., Kissell, D., Meller, J., Villanueva, J., Risma, K.A., Wei, Q., Klein, P.S. & Filipovich, A.H. (2011) Hypomorphic mutations in PRF1, MUNC13-4, and STXBP2 are associated with adult-onset familial HLH. *Blood*, **118**, 5794–5798.

Zhang, M., Behrens, E.M., Atkinson, T.P., Shakoory, B., Grom, A.A. & Cron, R.Q. (2014) Genetic defects in cytolysis in macrophage activation syndrome. *Current Rheumatology Reports*, **16**, 439–446.

Zhou, C., Xie, Z., Gao, L., Liu, C., Ai, J. & Zhang, L. (2015) Profiling of EBV-Encoded microRNAs in EBV-Associated Hemophagocytic Lymphohistiocytosis. *Tohoku Journal of Experimental Medicine*, **237**, 117–126.

Zoller, E.E., Lykens, J.E., Terrell, C.E., Aliberti, J., Filipovich, A.H., Henson, P.M. & Jordan, M.B. (2011) Hemophagocytosis causes a consumptive anemia of inflammation. *The Journal of Experimental Medicine*, **208**, 1203–1214.