Hemophagocytic Lymphohistiocytosis in children: Queen Rania Children's Hospital experience.

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ABSTRECT

Objectives: The aim of this study is to describe the clinical and laboratory features, etiological triggers, treatment and outcome of Hemophagocytic Lymphohistiocytosis (HLH) at single center.

Methods: Clinical features, laboratory findings, underlying causes, therapeutic options and outcomes of 42 patients with HLH who were diagnosed Queen Rania Children's Hospital, Amman, during the period January 2010 through September 2016 are described.

Results: Total number of cases were 42; 23 males (54.8%) and 19 females (45.2%), age was ranged from one month to 12 years. All patients met five to six criteria out of the eight HLH-2004 criteria: all patients had fever, splenomegaly, cytopenias and hemophagocytosis in the bone marrow examination; hyperferritinemia at time of presentation was seen in (95.9%), and proven CNS involvement in 10 patients (23.8%). A significant number of our cohort had oculocutaneous albinism phenotype (18 patients; 42.9%). Primary HLH was considered in 69%, while secondary or reactive HLH in 31%. All patients had received corticosteroids and cyclosporine as initial therapy, etoposide (VP-16) had been used only in 6 patients, rabbit antithymocyte globuline (ATG) in 5, and 10 patients (23.8%) received 11 allogenic hematopoietic stem cell transplantations (HSCT) as a curative treatment. The overall survival rate was 81%, HSCT group showed excellent outcome with survival rate of 90%.

Conclusion: Despite the lack of molecular diagnosis and some sophisticated tests used in diagnosis of HLH, we had diagnosed and successfully managed HLH in pediatric population. Further studies are required to improve awareness and outcomes in children.

Key words: Hemophagocytic lymphohistiocytosis, Underlying causes, Outcomes.

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Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a clinical syndrome characterized by uncontrolled hyper activated immune system due to impaired natural killer (NK) cells and cytotoxic T lymphocytes (CTL) function, which results in a life-threatening inflammatory reaction, multi-organ dysfunction, and hemophagocytosis in the reticulo- endothelial system [(1)]. HLH can be categorized as primary

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(genetic) and a secondary (reactive forms) Table I, the primary also called familial HLH (or FHL) when there is a family history of HLH or known underlying genetic defects. The secondary, reactive forms occur in the sittings of infections, malignancies, and autoimmune disorders (macrophage activation syndrome - MAS)[1,2,3]. HLH is a heterogeneous disorder, so the distinction between the primary and secondary entities may not be possible based on clinical settings, as both may be triggered by infections, although primary may present during early childhood but both can present at any age, and underlying genetic mutation is found in only 40% of all primary HLH patients [4, 5]. A set of diagnostic guidelines was first proposed by the Histiocyte Society in 1991and was revised in 2004 (Box I) based on common clinical, laboratory and histopathological findings [5]. HLH is a fatal disease in children and adults, the Histocyte Society in 2004 reported a 55% survival rate in treated patients, where prompt recognition and early treatment have contributed to outcome improvement [5]. Although the disease is rare in children, it has been estimated that tertiary care paediatric hospitals in North America should expect 1 case of HLH per 3000 inpatient admissions [6]. Given the rarity of HLH and that most of the current knowledge of the disease has relied on single-center case series specially in children, we conducted a retrospective analysis of the HLH in paediatric patients diagnosed and managed by Immunology Division at Queen Rania Children's Hospital, Amman, Jordanian Royal Medical Services. From this cohort we describe the clinical nd laboratory features, etiological triggers, treatment and outcome.

Table I: Hemophagocytic Lymphohistiocytosis Classification

Primary or Genetic Hemophagocytic Lymphohistiocytosis	**Subtype and genetic defect**
1. Familial Hemophagocytic Lymphohistiocytosis (FHL).	Type 1FHL: HPLH1, 9q21.3-q22 Type 2 FHL: PRF1, 10q21-22 Type 3 FHL: Munc13-4, 17q25 Type 4 FHL: STX11, 6q24.1 Type 5 FHL:STXBP2, 19p13.3-13.2
2. Immune Deficiency Syndromes	Griscelli syndrome type II (RAB27A) Chediak-Higashi syndrome (LYST) Hermansky-Pudlak syndrome type II (AP3B1) Wiskott–Aldrich syndrome (WAS) Severe combined immunodeficiency (IL2RG)
3. EBV-driven HLH	X-linked lymphoproliferative syndrome (XLP) Type 1: *SH2D1A* (*SAP*) Type 2: *BIRC4* (*XIAP*) IL-2–inducible T-cell kinase deficiency (ITK) CD27 deficiency (CD27) X-linked immunodeficiency with Mg2+ defect XMEN (MAGT1)
Secondary or Reactive Hemophagocytic Syndrome	
1. Infection-Associated hemophagocytic syndrome	Viral (Herpesvirus, HIV, Hepatitis, Enterovirus Parvovirus B19) Bacterial (Staphylococcus aureus, Salmonella typhi sp, Campylobacter sp, Rickettsia sp, Brucella sp, Mycoplasma sp, Mycobacterium tuberculosis) Fungal (Candida sp, Cryptococcus sp, Pneumocystis sp, Histoplasma sp, Aspergillus sp) Parasitic (Leishmania sp, Plasmodium sp, Toxoplasma sp)
2. Malignancy-associated hemophagocytic syndrome	Hematopoietic malignancies, Solid tumors
3. autoimmune disease - associated hemophagocytic syndrome (Macrophage activation syndrome)	Systemic onset juvenile idiopathic arthritis (Still disease), Systemic lupus erythematosus, Kawasaki disease

Box 1: The diagnosis of haemophagocytic syndrome is established by fulfilling one of the following criteria:

1. A molecular diagnosis consistent with haemophagocytic syndrome .
2. Diagnostic criteria of HLH fulfilled (Having five out of eight criteria of the following):

Clinical criteria:

1. Fever
2. Splenomegaly

Laboratory criteria:

3. Cytopenia (affecting more than two cell lineages, haemoglobin ≤9g/dL, <100 000 platelets per μL, neutrophils <1000 cells per μL)
4. Hypertriglyceridaemia (triglycerides ≥265 mg/dL) and/or hypofibrinogenaemia (fibrinogen ≤150mg/dL)
5. Low or absent natural killer cell cytotoxicity
6. Hyperferritinaemia (ferritin ≥500 ng/mL)
7. Elevated soluble CD25 (interleukin-2Rα chain ≥2400 IU/mL)

Histopathological criteria:

8. Haemophagocytosis in the bone marrow, spleen, or lymph nodes without evidence of malignancy

Methods

We reviewed the medical records of pediatric patients aged 0-14 years that had been diagnosed as HLH based on revised HLH-2004 Criteria, at Immunology and Rheumatology Division, Queen Rania Children's Hospital during the period January 2010 through September 2016. Demographic, clinical, and laboratory data were collected by standardized data collection form including the following data: age, gender, family history, consanguinity, fever duration, presence of hepatomegaly, splenomegaly, lymphadenopathy, known underlying disease, skin rash, treatment prescribed, and outcome. Laboratory data included: complete blood count, blood film, liver enzymes, serum ferritin, triglycerides, fibrinogen, C-reactive protein, erythrocyte sedimentation rate (ESR), lactate dehydrogenase, blood urea nitrogen, creatinine, prothrombin time, bone marrow aspirate and biopsy, blood and bone marrow cultures, cerebrospinal fluid (CSF) analysis in indicated patients, infections screen including the common causes of reactive HLH, and EBV by polymerase chain reaction (PCR) technique. Two laboratory diagnostic criteria were not tested, namely the soluble CD25 level and NK cell cytotoxicity, as they were not available at our facility. The hair shaft examination was reserved for hypopigmented primary immunodeficiency (PID) syndromes. This study was approved by the Jordanian Royal Medical Services Ethics committee (no. 44/6/3017).

Results

We reviewed the medical records for 42 pediatric patients who met HLH criteria. They were 23 males (54.8%) and 19 females (45.2%), age was ranged from one month to 12 years, with a median age at time of HLH diagnosis 3.0 years, 13 patients (31%) were below one year of age.

Clinical features:

All the 42 patients met five to six criteria out of the eight HLH-2004 criteria (of note two laboratory criteria were not tested for all patients; sCD25 level and NK cell cytotoxicity). The patients were either referred from other hospitals to our facility as pyrexia of unknown origin or to rule out primary immunodeficiency disorder, or they were known to have pre-existing illness, being followed by pediatric immunology and rheumatology teams at our hospital. Presenting clinical and laboratory features are summarized in Table II. All patients had fever, splenomegaly, cytopenias, and showed hemophagocytosis in the bone marrow examination. Ferritin level was >500 ng/mL at time of presentation in 40 patients (95.9%), while the reminder two patients showed hyperferritinaemia later during the disease course and 35 patients (83.3%) had serum ferritin >2000 ng/ml. All patients had either hypofibrinogenemia or hypertriglyceridemia, while 32 patients(76.2%) had both. Elevated transaminases had been observed in 19 patients (45.2%) associated with

hyperbilirubinemia in 8 patients (19.1%), one patient presented as fulminant hepatitis (referred from gastrointestinal service, when excluded infectious hepatitis profile). CSF examination was not feasible in all patients as most had marked thrombocytopenia and respiratory compromise so that lumber puncture could not be performed. Therefore we listed patients who developed clinical CNS manifestations with neuroimaging findings in addition to patients with pleocytosis, and proven CNS involvement was found in 10 patients (23.8%). A significant number of our cohort had oculocutaneous albinism phenotype (18 patients; 42.9%), 10 of them were diagnosed with Griscelli syndrome, and 8 with Chediak-Higashi syndrome on clinical grounds (the differentiation between the two was based on hair shaft examination Figure 1 and presence or absence of giant inclusions in polymorphonuclear neutrophils (PMNs) Figure 2; only 2 patients had the molecular diagnosis of RAB27A mutation).

Table II: Presenting clinical and laboratory features

	number	**percentage**
male	23	54.8%
female	19	45.2%
total	42	100%
age < 1 year	13	31%
fever	42	100%
splenomegaly	42	100%
cytopenias	42	100%
hypertriglyceridemia	39	92.9%
hyperferritinaemia	40	95.2%
hypofibrinogenemia	34	81%
hemophagocytosis in the bone marrow	42	100%
CNS involvement	10	23.8%
elevated transaminases	19	45.2%
hyperbilirubinemia	8	19.1%
oculocutaneous albinism phenotype	18	42.9%

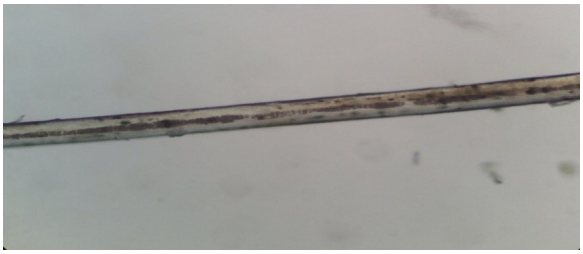

Fig 1a

Fig 1b

Figure 1a&b: Hair shaft microscopy (Griscelli Syndrome): large, clumped melanosomes in hair shafts secondary to pathology of melanocytes and keratinocytes.

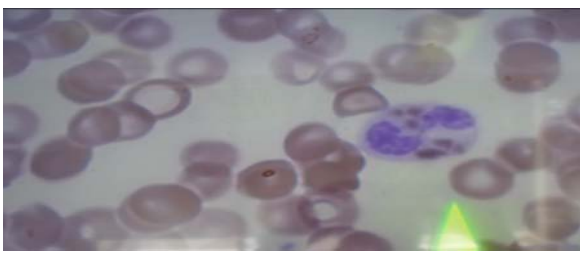

Fig 2a

Fig 2b

Figure 2a&b: Blood film (Chediack-Higashi Syndrome): giant inclusions in polymorphonuclear neutrophils (PMNs)

Causes:

Our approach to identify the underlying cause of HLH was based on following considerations: pre-existing illness (carries a risk for HLH), oculocutaneous albinism, connective tissue disease manifestations, family history of sibling death and/or similar phenotype, age at presentation, and response to treatment. Etiological causes and triggers of this cohort are listed in Table III. Primary HLH was considered in 29 patients (69%), and further sub-classified as following. Ten patients (23.8%) had Griscelli syndrome based on clinical phenotype, hair-shaft examination and absence of giant inclusions in PMNs, 8 (19%) Chediak-Higashi syndrome, based on clinical phenotype and presence of giant inclusions in PMNs, and 5 (11.9%) had EBV-driven HLH based on detection of EBV in plasma (by real-time PCR) and negative other infectious causes. We listed this category in the primary HLH because of the known association of EBV-driven HLH with mutations (e.g. *SH2D1A* (*SAP*), *BIRC4* (*XIAP*) etc.), and as the course of HLH in the 5 cases was not suggestive of a reactive form. One patient (2.4%) was diagnosed with severe combined immunodeficiency (T-B+NK+ phenotype) at age of 5 months, developed HLH and died during the first week of treatment before planned HSCT. Five further patients (11.9%) were considered likely as familial HLH based on young age at presentation (all <18 months), family history of unexplained sibling death (4 out of 5), treatment refractoriness and/or reactivating disease course (3 patients were refractory, 2 had frequent reactivations), and negative results of work-up for other PID. Secondary or reactive HLH was considered in 13 patients (31%). In 2 (4.8%) the bone marrow culture grew Brucella spp. and retrospective anti-Brucella antibody titre was high in both (1:640, 1: 1280). The patients were referred to infectious disease service for specific antimicrobial therapy, and HLH treatment was gradually weaned off (toward week eight of treatment). Five patients (11.9%) turned out to have underlying autoimmune disease, 3 had skin rash and lymphadenopathy, hepatomegaly, and later showed arthritis, fulfilling classification criteria of systemic onset juvenile idiopathic arthritis (SoJIA); other 2 female patients eventually fulfilled systemic lupus erythematosus (SLE) classification criteria. One 4-year male patient (2.4%) was diagnosed with B cell lymphoma on bone marrow biopsy and was referred to oncology service for specific HLH-Lymphoma treatment. We could not define a causative trigger or associated disease in 5 patients (11.9%), but we classified them as reactive HLH because they were healthy before HLH presentation, had no family history, all showed remission within the first 8 weeks, and never had reactivation during long-term follow up (mean 36 months).

Table III: Classification of our cohort

		number	percent (%)
primary	FHL (family history)	5	11.9
	GS	10	23.8
	CHS	8	19.0
	SCID	1	2.4
	EBV driven	5	11.9
	subtotal	29	69%
secondary	unknown	5	11.9
	Brucllosis	2	4.8
	SoJIA	3	7.1
	SLE	2	4.8
	malignancy	1	2.4
	subtotal	13	31%
Total		42	100%

Treatment:

Once the diagnosis of HLH was established, prompt treatment was started to achieve disease control and remission. Steroids and cyclosporine was the mainstay of therapy, and although the HLH-2004 protocol was our guidance in treatment, few patients got the whole treatment protocol Table IV lists the treatment options used in our patients). All patients had received corticosteroids and cyclosporine as initial therapy, either dexamethasone IV pulses based on HLH-2004 protocol or IV methylprednisolone pulses (dose ranged from 10-30 mg/kg/day) if patient did not respond to dexamethasone. Etoposide (VP-16) had been used only in 6 patients (14.3%) who were likely to be familial HLH; rabbit antithymocyte globulin (ATG) was used 5 (11.9 %) who were refractory to initial therapy with corticosteroids, cyclosporine with or without etoposide as a salvage therapy bridging to hematopoietic stem cell transplantation (HSCT). All patients as well received supportive therapy which included intravenous immunoglobulin (IVIG), cotrimoxazole, antifungal prophylaxis and gastroprotection. 10 patients (23.8%) received 11 allogenic HSCT as a curative treatment; 5 with Griscelli syndrome, 3 with Chediack Higashi syndrome, and 2 with refractory HLH who were classified as FHL, one of them received two transplants. All EBV-driven HLH were treated with rituximab (Anti-CD20 monoclonal antibody) in addition to steroids, cyclosporine and supportive therapy, and two patients needed mycophenolate mofetil (MMF) to control lymphoproliferation. Intrathecal treatment (methotrexate, steroids...) was used only in 3 patients (7.1%) who showed severe clinical CNS manifestations that outweighed the risk of intrathecal therapy in presence of marked thrombocytopenia. Patients with SoJIA received tocilizumab (Anti-IL-6 monoclonal antibody) to control systemic inflammatory manifestations, MMF was indicated in the two SLE patients, and anti-brucellosis treatment was used in the 2 cases with proved brucellosis.

Table IV: Treatment options in study cohort

	No	%
steroids + CsA	42	100.0
supportive therapy	42	100.0
HSCT	10	23.8
etoposide	6	14.3
ATG	5	11.9
rituximab	5	11.9
MMF	4	9.5
tocilizumab	3	7.1
intrathecal therapy (MTX, steroids)	3	7.1
specific antimicrobial therapy	2	4.8

Outcome:

Table V summarizes the outcome of our cohort. The overall survival rate was 81% (n=34) with a mean follow up of 27.4 months, survival rate in primary HLH was 72.4%. Disease remission was achieved in 73.8% (n=31) during induction therapy, while 7 patients (16.7%) were not responding to induction treatment (5 likely familial HLH, and 2 SoJIA). We treated refractory cases using IV methylprednisolone pulses, and as none of them showed complete response, salvage treatment using ATG was commenced in likely familial cases whilst the patients were prepared for HSCT, unfortunately only two patients survived and received HSCT as curative treatment. The two SoJIA patients treated with Tocilizumab showed good response and achieved remission. 12 patients developed disease reactivation (5 EBV-driven, 4 Griscelli syndrome, 3 Chediack Higashi syndrome), some of them had frequent reactivations necessitating long-term therapy. The overall mortality was 19% (8 patients) of which 3 likely familial HLH, 3 Griscelli syndrome, and 2 Chediack Higashi Syndrome. HSCT group showed excellent outcome with the survival rate of 90%, only one patient (a girl with Griscelli Syndrome) experienced transplant related mortality. 20 patients including 7 transplanted patients showed long-term remission with a mean follow up of 23.7 months.

Table V: Outcome

	No	%
alive	34	81.0
responders	31	73.8
non-responders (refractory)	7	16.7
disease reactivation(s)	12	28.6
long-term remission	20	47.6
mortality	8	19.0

Discussion

Hemophagocytic lymphohistiocytosis (HLH) is a rare often fatal disorder affecting both children and adults. The annual incidence of primary HLHis estimatedat 0.12 per 100,000 children per year in Sweden, around 1:50,000 live born, but it is believed that these figures are strongly underestimated, the overall HLH rate would be higher if secondary HLH is included as there are no published incidence rates for secondary HLH [(5)].However, recent reports described that over the years, increased recognition of HLH in general indicates increasing physicians awareness about HLH. [(7)] Up to our best knowledge this is the first report describing HLH clinical features and outcome in Jordanian children. Despite the limited laboratory facilities in diagnosis of HLH that are commonly feasible to many institutions worldwide, like NK cell activity assessment, soluble CD25 measurement, and molecular tests, HLH diagnosis was possible in 42 cases at Queen Rania Children's Hospital based on fulfilling five to six clinical and laboratory diagnostic criteria of the HLH-2004 protocol. Age ranged from one month to 12 years, a median age at time of diagnosis was 3.0 years, and around one third of our patients were less than one year of age. In comparison to a large series from Italian registry of HLH [(7)] reporting 500 cases over 25 years, where vast majority were pediatric patients (91.2%) with median age of 2.2 years and 29% below one year, a higher median age in our study could reflect delayed diagnosis of HLH. The younger the age of onset the higher is the possibility of primary HLH, and two third of Italian cohort who were below one year of age had familial HLH. Clinical presentations of our patient were comparable to other reports [(8, 9, 10)].We report on one case presenting as fulminant hepatitis initially managed by pediatric gastroenterologist, classified as reactive HLH, as a trigger could not be retrieved. Others had reported severe hepatitis as a presentation of HLH, most of these cases were due to autoimmune disease like SoJIA or SLE [(9)]. Interestingly, while elevated liver transaminases with or without hyperbilirubemina is a common laboratory finding in pediatric HLH rangeing from 70-100% [(11,12)], in our cohort we saw this in less than half of the patients. We found that all of the patients showed hemophagocytosis in the bone marrow, which was a common findings (75-95%) in many reports [(8,11,12,13)], although Cetica *et al* found hemophagocytosis only in 40% of the Italian cohort[(7)]. The authors recommended withdrawing this from diagnostic criteria, contrary to Fardet and co-workers who validated a diagnostic score set of clinical, biologic and cytologic variables for reactive HLH called Hscore[(14)] where the presence of hemophagocytosis has a significant weight. However, the absence of hemophagocytosis does not exclude the disease and it may be a late sign which could explain why it was a universal finding in our cohort even in the secondary cases, as we received most of the patients late. Proven CNS involvement was observed in 23.8%; clinically evident and confirmed by neuroimaging and/or CSF examination. CNS features in pediatric HLH range from 19-47%, and CSF findings in more than 45% even in the absence of clinical features indicate subclinical CNS involvement [(11)]. CSF examination in our cohort was limited by marked thrombocytopenia and/or respiratory compromise in the majority of the patients. One of the limitations of our cohort was no molecular diagnosis of familial HLH. Our approach in classification of HLH patients was depending on a set of clinical and laboratory criteria, adopted from published reports. In the recent Sweden report, authors defined primary HLH in patients who met at least five of the eight criteria according to the HLH-2004 protocol, diagnosed at age <15 years, with clinically severe HLH requiring HSCT or causing death, and they considered patients with a relapse-free survival >1 year off

treatment as most likely having secondary HLH [8]. Other report from India defined possible familial HLH in children if there was a positive family history, when HLH has multiple relapsing courses, and/or prominent CNS involvement at disease onset [15]. Although unavailability of molecular diagnosis should not delay initial treatment of HLH, it has importance in the genetic counseling and screening of siblings, and in making the decision for HSCT as curative treatment. This would therefore , suggests that most, if not all, young pediatric HLH patients should undergo full genetic testing if at all possible [16]. 69% of our cohort was classified as primary HLH; two thirds of them had oculocutaneous albinism phenotype with clinical diagnosis of Griscelli syndrome (GS) and Chediak-Higashi syndrome (CHS) at 34% and 28%, respectively. GS and CHS are hypopigmentation disorders of hair, skin, and eyes associated with immunodeficiency and neurological manifestations secondary to impairment in the function and trafficking of secretory lysosomes. HLH, also known as "accelerated phase" of the disease, affects both CHS (85% of patients within the first decade) and GS type 2 (the majority of patients in the first year of life) [17]. In our cohort, 8 cases (44%) were treated by HSCT, which is the most effective treatment of accelerating phase of CHS and GS [18, 19]. EBV-driven HLH has variable clinical phenotypes, ranging from mild self-limited disease to fulminant fatal HLH needing HSCT, and EBV is the most common infectious trigger of HLH. The incidence is relatively high in Asian population, it accounts up to 40% of all HLH in Japan, and can occur in the settings of genetic HLH and in apparently healthy children as well [20].Whilst genetic testing should be sought to differentiate different EBV-associated HLH disorders, we could not exclude underlying genetic defect in the five cases of EBV-HLH in our cohort. As all had severe disease and three had a relapsing course, we classified them as primary HLH. Thirteen cases were classified as secondary or reactive HLH which accounted for 31% of total HLH cases, which is less than reported in Italian registry study where authors used the term sporadic HLH in 56% with respect to non-familial cases in whom they did not find a known mutation of primary HLH[7]. In a recent report in pediatric population from China, Wang *et al* reported secondary HLH in 55% of total HLH [21]. Lower secondary HLH percentage of our cohort could be explained by the fact that 42.8% of our patients had Griscelli and Chediack Higashi Syndromes. In George MR [22] review of HLH etiologies in adults and children, the conditions associated with secondary HLH included viral infections (29%), other infections (20%), malignancies (27%), rheumatologic disorders (7%), and immune deficiency syndromes (6%). We reported only few infections in our cohort which could be explained by the nature of patient selection and referral to our service primarily to rule out PID or rheumatological disorders. The 5-year probability of survival reported by HLH-94 report was only 55%, but the advances in HLH recognition and treatment have improved overall survival with many reports showing 60-85% overall survival in different HLH groups[7,8,10,20,21]. The overall survival was favourable in our cohort (81%) and 72.4 % in primary category; patients were offered prompt treatment based on HLH-2004 protocol, and specific treatment for specific subtypes (e.g. rituximab for EBV-driven HLH, conventional and biological disease modifying anti-rheumatic drugs for auto-immune diseases), including HSCT for primary and refractory cases.

Conclusion

Despite the lack of molecular diagnosis and some sophisticated tests used in diagnosis of HLH, we had diagnosed and managed HLH cases in pediatric population. HLH in young children has different clinical and laboratory presentation compared to adults; in addition there are inherited pediatric disorders that might present or be associated with HLH. Published data in pediatric HLH are still limited, and further studies are needed to increase awareness among pediatricians for early recognition and referrals to specialized centers, for better HLH classification and for more treatment protocols for refractory cases

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