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Haemophagocytic Lymphohistiocytosis in an Infant: Important Aspects in Management

WH ***Hui,*** DKK ***Ng,*** KL ***Kwok,*** YY ***Lam***

Abstract Haemophagocytic lymphohistiocytosis (HLH) is a disorder of immune dysregulation that carries a poor prognosis. We reported a case of HLH in an infant that had a complete remission after receiving intravenous immunoglobulin (IVIG) and highlighted the important aspects in the management of such disease. Diagnosis of HLH should always be considered early in patients with unremitting fever, hepatosplenomegaly and cytopenias. Neurological involvement is common and lumbar puncture is recommended in all cases if clinical condition allows. NK cell function should be checked in all cases and helps to differentiate primary and secondary HLH. IVIG, corticosteroid, cyclosporine A and etoposide have all been suggested as treatment options of HLH. The HLH protocol should be started without delay for severe disease or primary HLH. For mild non-familial disease, IVIG had been suggested by some authors as the initial treatment although confirmed evidence is still lacking. Further studies are necessary to compare the efficacy of different treatment options. It is hoped that early diagnosis and intervention will increase the chance of survival.

Key words Haemophagocytic lymphohistiocytosis; Infant; Intravenous immunoglobulin; Management

Case Report

An 8-month-old girl was admitted on day 9 of fever with maculopapular rash over the face and trunk. Her general condition was good. There was hepatomegaly, 4.5 cm below costal margin with no splenomegaly, and shotty post-auricular lymph nodes. White cell count was 18.8 x 10^9/L which was lymphocyte predominant. Neutrophil, haemoglobin and platelet were normal. Liver function showed a raised alanine aminotransferase (ALT) (575 IU/L) and aspartate aminotransferase (AST) (228 IU/L). Albumin was 31 g/L. Clotting profile was normal with prothrombin time (PT) 12.7 sec, INR 1.08 and activated partial thromboplastin time (APTT) 29.5. D-dimer was 452 ng/ml which was normal (Normal range: <500 ng/ml) Echocardiogram was normal.

Patient became anaemic with haemoglobin 7.2 g/dL 5 days after admission and was admitted to paediatric intensive care unit. Her vital signs were stable. The rash also progressed to involve the limbs. The liver was enlarged to 7 cm and the spleen to 4 cm. Neutrophil dropped to 0.9 x10^9/L and platelet to 116 x 10^9/L. Liver enzymes were further elevated with AST 987 IU/L and ALT 1487 IU/L. PT was prolonged to 16.2 sec and INR to 1.36. APTT was normal. Lowest albumin was 19 g/L. Transfusion of packed cells, fresh frozen plasma and albumin were given. Neurological status remained stable.

Bone marrow examination performed on day 5 of admission showed mild reactive haemophagocytosis with mildly increase in histiocytes to about 1% of all cells,

Department of Paediatrics, Kwong Wah Hospital, 25 Waterloo Road, Kowloon, Hong Kong, China

WH **Hui** （許慧嫻） *MRCPCH*
DKK **Ng** （吳國強） *MD, M Med Sc*
KL **Kwok** （郭嘉莉） *FRCP, FHKAM(Paed)*
YY **Lam** （林琬瑜） *FRCP, FHKAM(Paed)*

Correspondence to: Dr WH **Hui***

*Department of Paediatrics, Caritas Medical Centre, 111 Wing Hong Street, Shamshuipo, Kowloon, Hong Kong, China

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occasional haemophagocytic histiocytes and no evidence of malignancy. Further investigations showed a raised triglyceride (1.63 mmol/L) (normal range: 0.40-1.24 mmol/L), a raised lactate dehydrogenase (LDH) (1492 IU/L) (normal range: 190-420 IU/L), a low fibrinogen (1.6 g/L) (normal range: 2.0-4.0 g/L) and a high ferritin (1179 ng/ml) (normal range: 7-283 ng/ml). The constellation of fever, splenomegaly, bi-cytopenia, hyprferritinaemia and haemophagocytosis in the bone marrow confirmed the diagnosis of haemophagocytic lymphohistiocytosis (HLH).[1] One dose of intravenous immunoglobulin (IVIG) infusion at 2 g/kg was given on the same day. Deferversence occurred after IVIG and she was afebrile 3 days afterwards.

Infection screening including IgM against Ebstein-Barr virus (EBV) VCA, rubella, measle and hepatitis A virus, and nasopharyngeal aspirate for respiratory viruses including influenza A, B, and parainfluenza type 1-3 virus were all negative. Blood culture showed no growth. Immunoglobulin levels and rheumatological markers were normal. There was no family history of similar presentation.

The child was transferred to the general ward after her fever had subsided for 3 days. Liver was 4 cm and spleen was 2 cm. White cell count, platelet and neutrophil count returned to normal. Haemoglobin was 8.9 g/dL. Liver enzymes showed a decreasing trend with AST 69 IU/L and ALT 213 IU/L. Ferritin dropped to 217 ng/ml. Triglyceride increased further to 2.44 mmol/L (normal range: 0.6-2.1 mmol/L) which fulfilled another criteria of the diagnosis of HLH.[1,2]

Patient was discharged after 13 days of hospitalisation. Two weeks after discharge, liver enzymes decreased to AST 65 IU/L and ALT 113 IU/L. Haemoglobin returned to 10.6 g/dL. Other parameters had all returned to normal. At three weeks after discharge, liver size and liver enzymes also became normal. Natural killer (NK) cell function was checked in the recovery stage and was normal. On subsequent follow-up at 16 months of age, the girl was well with normal growth and development.

Discussion and Literature Review

HLH is a rare disease of disturbed immune regulation that involves multiple organs and carries a high mortality. The disease affects both children and adults. The manifestations are caused by hypercytokinaemia and organ infiltration by phagocytosing histiocytes due to an ineffective, uncontrolled immune response secondary to an inherited or acquired defective NK function.[3] The incidence of familial HLH (FHLH) in Sweden was about 1.2/1,000,000 children/year (around 1:50,000 live born).[4] The incidence that included secondary HLH would be higher.

HLH can be caused by various infections, of which viral infections are the most common. EBV is the most frequent cause of virus-associated HLH, especially in Asia.[3] Other viruses including cytomegalovirus (CMV), adenovirus and influenza A had been reported to cause HLH.[5] Rheumatic diseases and malignancies can also cause HLH.[3] Rheumatic disease-associated HLH, sometimes called macrophage activation syndrome, occurs most commonly in systemic juvenile idiopathic arthritis,[6] but may occur in other rheumatic disorders. Primary or familial HLH is an autosomal recessive disease. 70-80% of the cases had their onset in their first year.[3] Since primary or familial HLH is often triggered by an infection, the presence of an infection does not rule out FHLH.[3]

Genetic studies have revealed mutations in the perforin gene in 20-40% of patients with primary HLH.[7] In FHLH caused by perforin 1 gene (PEF1) mutations, there is deficiency of perforin which is a key membranolytic protein expressed in NK cell. Its function is to form pores in membranes of target cells, thus allowing entrance of granzymes which triggers apoptosis.[1] With deficiency of perforin, NK cell function is abnormal. Killing of infected cells by NK cell became ineffective. The source of antigen stimulation persisted and thus produced a persistent uncontrolled activation of immune cells, leading to hyperinflammatory state with increase production of cytokines that stimulate macrophages.[8] Recently , it has been reported that mutations in two other genes, hMunc13-4 and syntaxin 11 also cause HLH.[7] Munc13-4 is required for priming of cytolytic granules for membrane fusion and exocytosis while syntaxin 11 is involved in vesicle trafficking.

Diagnosis

Fever, hepatosplenomegaly and cytopenia are typical findings of HLH.[3] Other common findings include hypertriglyceridaemia, coagulopathy, hypofibrinogenaemia and hyperferritinaemia.[3] The development of neurological disease is associated with poor outcomes.[9] Progressive cytopenias leading to bacterial or fungal sepsis, bleeding and cerebral dysfunction are the terminal events.[3]

The disease is often unrecognised at early stage and thus affected children may receive suboptimal treatment, leading

to high mortality. Many clinical manifestations of HLH also resemble those of an overwhelming viral infection which is considered to be much more common. Since HLH carries a high mortality, it should always be considered early in the differential diagnosis of pyrexia of unknown origin.

Diagnostic guidelines for HLH presented in 1991 by the HLH study group of the Histiocyte Society was shown in Table 1. It required all 5 criteria fulfilled to confirm the diagnosis. Based on the observation that some patients actually develop one or more of the diagnostic criteria late during the course of the disease[2] and the added knowledge on molecular diagnosis, the Society had revised the diagnostic guideline in 2004.[1,3] Three additional criteria is included and the diagnosis is established if either molecular diagnosis consistent with HLH is confirmed or 5 out of the 8 criteria listed in Table 2 fulfilled.[1] Elevated tranaminases, bilirubin, LDH more than 1000 IU/L and cerebral symptoms with cerebrospinal fluid (CSF) showing moderate pleocytosis and/or elevated protein were included as supportive evidence and evidence of haemophagocytosis

Table 1 The 1991 diagnostic criteria for HLH[2]

Initial diagnostic criteria (to be evaluated in all patients with HLH)
Clinical criteria
1) Fever
2) Splenomegaly
Laboratory criteria
3) Cyopenias (affecting ≥2 of 3 lineages in the peripheral blood: Haemoglobin (<9 g/dL), platelet (<100 x 10^9/L), neutrophils (<1.0 x 10^9/L) (In infants <4 weeks: Haemoglobin <10 g/gL)
4) Hypertriglycridaemia and/or hypofibrinogenaemia (fasting triglycrides ≥3.0 mmol/L , fibrinogen ≤1.5 g/L)
Histopathologic criteria
5) Haemophagocytosis in bone marrow or spleen or lymph nodes. No evidence of malignancy.

Table 2 Diagnostic criteria for HLH (HLH-2004)[1]

A) Initial diagnostic criteria (to be evaluated in all patients with HLH)
Clinical criteria
1) Fever
2) Splenomegaly
Laboratory criteria
3) Cyopenias (affecting ≥2 of 3 lineages in the peripheral blood: Haemoglobin (<9 g/dL), platelet (<100 x 10^9/L), neutrophils (<1.0 x 10^9/L) (In infants <4 weeks: Haemoglobin <10 g/gL)
4) Hypertriglycridaemia and /or hypofibrinogenaemia (fasting triglycrides ≥3.0 mmol/L , fibrinogen ≤1.5g/L)
Histopathologic criteria
5) Haemophagocytosis in bone marrow or spleen or lymph nodes. No evidence of malignancy.
B) New diagnostic criteria
6) Low or absent NK-cell activity (according to local laboratory reference)
7) Ferritin ≥500 microgram/L
8) Soluble CD25 (i.e. soluble IL-2 receptor) ≥2400 U/ml

is not an essential diagnostic criterion.[1] The introduction of the 3 additional criteria would facilitate more early diagnosis and treatment, which would probably improve the chance of survival.

Symptomatic neurological involvement developed in 75% of patients.[9] CSF abnormalities can occur without symptoms.[3] In two retrospective studies on HLH, only around 1 in 4 patients underwent lumbar puncture.[6,10] The proportion that underwent neuroimaging was even lower.[6] It seemed that neurological investigations are often overlooked in HLH. Lumbar puncture was also not considered in our patient as she had no abnormal neurological signs. Lumbar puncture is actually recommended in all cases if clinical condition allows.[1] It can provide supportive evidence for the diagnosis and diagnose neurological involvement which require treatment with dexamethasone that penetrates the blood brain barrier well.[11] MRI brain is also recommended in patients with neurological symptoms to look for signs of inflammation which is present in nearly half of the patients at initial presentation.[12]

Although our patient was in infancy which is the commonest time of onset in primary or familial HLH, the normal NK cell function, the absence of family history, the mild disease severity and the good outcome suggested that her disease was secondary rather than primary. In primary or familial HLH, the NK cell function is usually markedly decreased or absent[1] and it is likely to be caused by an underlying genetic defect. In patients with infection-associated HLH, the NK cell activity may also be low. The defective NK cell function in infection-associated HLH is acquired and likely to be transient. NK cell activity should be checked in all patients[1] and repeated in the convalescence phase if the first level is low. Evidence of normal NK cell function suggests that HLH is secondary rather than primary. Molecular study is indicated if the NK cell function is persistently low or when there is family history of similar presentation. Differentiating primary from secondary HLH is important, although not always possible, as this would help to refine treatment selection which would be discussed later.

In relation to possibility of EBV infection, only EBV VCA IgM was checked in our patient. In EBV-associated HLH, EBV antibodies are not always present as the serology may be affected by the abnormal immune response in HLH. Therefore EBV genome should be detected by PCR or by *in-situ* hybridisation on biopsies of bone marrow if EBV serology was negative.[5] Cytomegalovirus (CMV) was not studied in our patient. Demonstrating evidence of CMV infection is also important as it had also been reported to be associated with HLH in children[5] and antiviral therapy for CMV is available. CMV infection can also be confirmed by detecting CMV DNA using PCR.

Treatment

Some diagnostic criteria may develop late during the course of the disease,[2] therefore definitive therapy may have to be commenced on strong clinical suspicion. Patients who are critically ill with unremitting fever, progressive bi- or pancytopenia, clotting derangements or liver failure, and who has familial or primary HLH should be started on the HLH protocol which includes etoposide, dexamethasone and cyclosporine A.[3] The 5-year survival rate after treatment with HLH-94 (treatment protocol developed by the Histiocyte Society in 1994) was 55%[11] which represents a large improvement compared to a 5-year survival rate of 22% in one previous study.[13] In HLH-94, cyclosporine was introduced after 8 weeks of initial treatment. Since most of the death occurred in the first 2 months of therapy,[11] one major change in HLH-2004 protocol which is the treatment protocol under study by the Histiocyte Society, was to introduce cyclosporine at the onset of therapy.[1] The frequency of opportunistic infections while receiving etoposide was reduced by early use of cyclosporine in one study.[14] The mechanism is probably through ameliorating etoposide-induced or cytokine-induced neutropenia.[14] For presumed infection-associated HLH, IVIG has been suggested as an initial treatment option for mild disease[3,14,15] Initiating etoposide in severe disease should not be delayed,[14,15] especially in EBV-associated HLH as mild cases may rapidly progress into a life-threatening course.[3] The results of studies on different treatment regimens are summarised in Table 3. A 4-year overall survival rate of 78.3% had been reported in a study on the use of etoposide in EBV-associated HLH. Survival rate at 4 years with early introduction of etoposide before 4 weeks from diagnosis was 90.2% compared to 56.5% with introduction of etoposide after 4 weeks.[14] The risk of development of secondary leukaemia in HLH patients following etoposide is limited and acceptable considering its positive therapeutic effects.[11] For rheumatic disease-associated HLH, high dose corticosteroids (≥2 mg/kg body weight) and

Table 3 Summary of study results on different treatment of HLH

	Henter et al, 2002[11]	**Imashuku et al, 2001[14]**	**Imashuku et al, 1999[18]**	**Chen et al, 1998[19]**	**Chen et al, 1995[20]**	**Stéphan et al, 2001[6]**
Study	Prospective study	Restrospective study	Observational study	Observational study	Observational study	Restrospective study
Aetiology	Heterogenous	EBV	EBV	Heterogenous	Virus-associated HLH	Rheumatic-disease associated HLH
Sample size	113	47	17	22	17	24
Treatment	HLH-94	Etoposide started <4 wks (n=30) vs started later or not at all (n=17)	Mainly HLH-94 / etoposide + steroid	13/22: IVIG→ etoposide + prednisolone 4/22: IVIG + etoposide + prednisolone 5/22: Etoposide and prednisolone	IVIG (IVIG2 or IVIG5) and/or etoposide IVIG2: 1g/kg/dx2d IVIG5: 400 mg/kd/dx5d	IV steroid ± cyclosporin
Outcome	Survival at 3 years: 55% ± 9%	Survival at 4 years: 90.2% ± 6.9% vs 56.5% ± 12.6% ($p<0.01$) Overall survival at 4 years: 78.3% ± 6.7%	All CR except one relapse	Survival at 5 years: 40.9% ± 10.5% Disease free survival: 36.4% ± 14.5	2/9 IVIG alone: CR 8/17: CR	15/24: respond to steroid 7/9 failed steroid respond to CSA 2/9 died- one had transient improvement with IV steroid + etoposide
Remarks		10/12 initially receiving IVIG + steroid ± cyclosporine (CSA) require switch to etoposide		IVIG vs no IVIG: ns	IVIG2 vs IVIG5: ns	
		Risk of opportunistic infections: Early etoposide + CSA vs late/no CSA: 0 vs 16/20 (ns)				

n=no. of patients; ns: non-significant; CR: complete respond

cyclosporin A were recommended.[5] Supportive care including prompt treatment of infection, correction of fluid-electrolyte imbalance and coagulation derangement are also important. For children with familial or primary HLH, cure can only be achieved by stem-cell transplantation.[11]

Our patient had made good progress after receiving IVIG. It is difficult to ascertain whether the symptom resolution is related to the effect of IVIG or it had happened spontaneously. Spontaneous resolution of secondary HLH have been reported.[16] The good outcome of our patient is probably related to the mild disease severity. There are very few published reports about children with HLH responding to IVIG and there are no confirmed benefits in studies that include IVIG as one of the treatment regimens. The use of IVIG against EBV-[3] and rheumatic disease-associated HLH[6] were also unimpressive. For mild diseases, IVIG had been recommended as the initial treatment, [3,10,12,14,15] while preparing to switch to etoposide-containing regimens at first signs of failure.[3,14,15] There are no consensus towards the dose and duration of IVIG therapy. High dose IVIG was recommended in one review[12] and was successful in inducing improvement within 24-72 hours in one adult case series.[17] One study showed no significant difference in the treatment effect with IVIG 400 mg/kg/day given for 5 days and 1 g/kg/day given for 2 days.[10]

Conclusion

Over the past 25 years, survival of children with HLH has improved from 5% at 1 year to greater than 50% 3-5 years after diagnosis.[12] The diagnosis should be considered early in patients with unremitting fever, hepatosplenomegaly and cytopenias. Treatment should be started on strong clinical suspicion. Neurological involvement is common and lumbar puncture is recommended in all cases if clinical condition allows. NK cell function should be checked in all cases of HLH and helps to differentiate primary and secondary HLH.

Intravenous immunoglobulin (IVIG), corticosteroid, cyclosporine A and etoposide have all been suggested as treatment options of HLH. What constitutes the best treatment approach is still controversial. The HLH protocol should be started without delay for severe disease or primary HLH. For mild non-familial disease, IVIG had been suggested by some authors as the initial treatment although confirmed evidence is still lacking. Further studies are necessary to compare the efficacy of different treatment options. It is hoped that early diagnosis and intervention will increase the chance of survival.

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