MEDICAL PRACTICE

Haemophagocytic lymphohistiocytosis in Hong Kong children

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Haemophagocytic lymphohistiocytosis is a rare but potentially fatal disease. Diagnosing this disease may be difficult and is often delayed because the clinical presentation mimics other conditions like severe sepsis, hepatic failure and malignancies. We reviewed the clinical presentations, response to treatment, and outcomes of children diagnosed with haemophagocytic lymphohistiocytosis from 1991 to 2006 in a Hong Kong tertiary paediatric haematology centre. All patients had typical presentations with prolonged fever, organomegaly, and pancytopaenia. Six children had hepatic dysfunction and two had neurological symptoms. The median time from disease onset to diagnosis was 21 days. Elevated serum ferritin levels and evidence of haemophagocytosis in bone marrow examinations aided diagnosis. The overall mortality was 57%. Three patients who presented in the first few years studied had relatively long lag times between disease onset and definitive treatment; all died of active disease. Three patients diagnosed more recently were given timely treatment using the haemophagocytic lymphohistiocytosis–94 protocol of etoposide and dexamethasone, with or without cyclosporin. All three achieved remission, but two had a recurrence and one died during the recurrence.

Introduction

Haemophagocytic lymphohistiocytosis (HLH) is a rare but potentially fatal disease. It is a disorder of macrophage-related cell proliferation, leading to a hyper-stimulated yet ineffective immune response that causes erythrophagocytosis and tissue damage.[1,2]

The clinical hallmarks of HLH are prolonged fever, massive hepatosplenomegaly and cytopaenia, but the presentation can be highly variable. Patients may also present with neurological symptoms, jaundice, lymphadenopathy and skin rashes. Hypertriglyceridaemia, hypofibrinogenaemia, liver dysfunction, and elevated ferritin are common laboratory findings. A bone marrow examination may demonstrate the presence of haemophagocytosis.

Diagnosing HLH may be difficult and is often delayed because the clinical presentation mimics other conditions like severe sepsis, hepatic failure and malignancies. Diagnostic guidelines devised by the Histiocyte Society are available. Untreated HLH has a high mortality rate but effective treatment with etoposide (VP-16), dexamethasone, and cyclosporine has been shown to improve survival significantly.

The aim of this case series was to share our local experience with the disease to heighten physicians' awareness of this diagnostic entity. We believe that early recognition and prompt initiation of treatment will improve the prognosis.

Key words
Child; Diagnosis, differential; Histiocytosis, non-Langerhans-cell; Treatment outcome

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Methods

The case notes of patients diagnosed with HLH between 1991 and 2006 at Lady Pao Children's Cancer Centre, Prince of Wales Hospital, a tertiary paediatric haematological and oncology centre in Hong Kong were retrospectively reviewed. The diagnosis of HLH was based on criteria described by the Histiocyte Society (Box).[3] These criteria were applied retrospectively to patients diagnosed before the guidelines were available.

Results

Seven patients were diagnosed with HLH during the 15-year period. The median age of onset was 5 years with a range of 16 months to 10 years. Most (six) were female. The median time from disease onset to diagnosis was 21 days, with a range of 10 to 60 days. There was a decreasing trend in time required to establish the diagnosis as cases became more recent. All patients were considered to have secondary HLH as none had parental consanguinity or a family history of haemophagocytic disorders at either presentation or follow-up.

Clinical features

All patients had fever and hepatomegaly. Splenomegaly was present in all but one patient. Four patients also had lymphadenopathy, skin rashes, and jaundice. The prevalence of neurological symptoms was 29% (Table 1). One patient had somnolence on presentation and another had seizures during a recurrence.

Laboratory investigations

All patients had pancytopaenia. Ferritin was grossly elevated (range, 3971-15120 μg/L) in all four patients in whom the level was checked on presentation. Hypertriglyceridaemia was found in four of five patients. Abnormal liver functions were common. All patients had evidence of haemophagocytosis in bone marrow samples taken on presentation (Table 2).

Two cases of Epstein-Barr virus (EBV)–associated HLH were identified (patients 4 and 6). Both had immunoglobulin M specific for EBV and an absence of antibodies to the EBV nuclear antigen. None of the others had any infections identified by cultures and serology performed on presentation. Natural-killer cell (NK cell) activity levels were only available in two patients. Low NK cell activity was detected during a recurrence (patient 5). Patients 5 and 6 had perforin gene mutation studies but both were negative.

Treatment and outcomes

Five out of seven patients received chemotherapy. Treatment was initiated late for three of the earlier patients, and all three died (patients 1, 3, and 4). The mean duration from disease onset to treatment was 25 days for patients who died but was only 13.5 days for the patients diagnosed more recently who survived (patients 5 and 6). It took 60 days to establish the diagnosis in patient 2. Her condition was so critical she was deemed unfit for chemotherapy and died before definitive treatment could be initiated. Patient 7 achieved a spontaneous recovery. Etoposide was the mainstay of therapy. Two earlier patients (patients 1 and 3) received VP-16 in conjunction with daunorubicin, cytarabine, and methylprednisolone, respectively. Both failed to achieve a clinical response at 2 to 4 weeks and died. Three patients (patients 4, 5, and 6) were treated according to the HLH-94 protocol with VP-16 and dexamethasone with or without

香港兒童的嗜血細胞性淋巴組織細胞增生症

嗜血細胞性淋巴組織細胞增生症是一種罕見但可致命的疾病。其臨床徵狀與嚴重膿毒病、肝衰竭和癌症等酷似，所以不容易被診斷，或往往會被延遲診斷。本文回顧1991到2006年間於香港一所三級兒科血液學中心診斷為嗜血細胞性淋巴組織細胞增生症的兒童病例，包括發病徵狀、患者對治療的反應和效果。所有患者均有長期發熱、內臟巨大和全血球缺乏的典型徵狀。六名患者出現肝功能失調，兩名則有與神經系統有關的症狀。發病到確診的平均時間為21天。上升的血清鐵蛋白水平和透過骨髓檢查驗出的噬血細胞現象均有助診斷。整體死亡率為57%。在研究的首數年，有三名患者發病後需頗長時間才開始正式治療，後全部死於重症；另外三名患者在有或無使用環孢靈的情況下，利用依托泊甘（VP-16）和地塞米松，配合1994年嗜血細胞性淋巴組織細胞增生症治療方案確診，並作即時治療，全部病情皆得以舒緩，但兩名患者及後復發，其中一名於復發期間死亡。

Box. Diagnostic guidelines for haemophagocytic lymphohistiocytosis (HLH)[3]

The diagnosis of HLH can be established if one of either 1 or 2 below is fulfilled:
1. A molecular diagnosis consistent with HLH
2. Diagnostic criteria for HLH fulfilled (5 out of 8 criteria below):

Clinical criteria
- Fever
- Splenomegaly

Laboratory criteria
- Cytopaenias
  Affecting ≥2 of 3 lineages in the peripheral blood: haemoglobin (<90 g/L), platelets (<100 x10^9 /L), neutrophils (<1.0 x10^9 /L); in infants <4 weeks: haemoglobin <100 g/L
- Hypertriglyceridaemia (fasting triglycerides ≥3.0 mmol/L [ie ≥265 mg/dL]) and/or hypofibrinogenaemia (fibrinogen ≤1.5 g/L)
- Low or absent NK-cell activity
- Ferritin ≥500 μg/L
- Soluble CD25 (ie soluble IL-2 receptor) ≥2400 U/mL

Histopathological criteria
- Haemophagocytosis in bone marrow or spleen or lymph nodes (no evidence of malignancy)

TABLE 1. Presenting clinical features

Case No. (year of diagnosis)	Age (years)	Fever	Hepatomegaly* (cm)	Splenomegaly* (cm)	Jaundice	Rash	Lymphadenopathy	Neurological symptoms
1 (1991)	6	Yes	5	5	Yes	Yes	Yes	No
2 (1993)	9	Yes	3	7	No	No	No	No
3 (1995)	1	Yes	6	4	No	Yes	Yes	Somnolence
4 (2001)	10	Yes	5	6	Yes	No	No	Seizure†
5 (2002)	5	Yes	5	0	Yes	Yes	Yes	No
6 (2002)	2	Yes	9	5	Yes	Yes	Yes	No
7 (2005)	5	Yes	4	2	No	Yes	No	No

* Enlargement of liver or spleen refers to centimetres below the costal margin in the mid-axillary line
† Seizure during recurrence

TABLE 2. Laboratory findings*

Case No.	Hb (g/L)	ANC (x10^9 /L)	Plts (x10^9 /L)	Fibrinogen (g/L)	TG (mmol/L)	Ferritin (μg/L)	Bilirubin (μmol/L)	ALT (IU/L)	CSF	Haemophagocytosis
1	82	0.7	51	NA	NA	NA	35	209	Normal	BM
2	86	0.1	36	NA	NA	NA	31	131	NA	BM
3	94	0.6	36	NA	NA	NA	46	459	Normal	BM, LN
4	93	0.6	55	NA	4.06	6003	30	216	Normal	BM
5	67	0.8	46	2.3	8.08	15120	279	548	Normal	BM
6	86	0.6	3	1.11	3.3	9037	98	1604	Normal	BM
7	97	2.2	33	2.07	2.35	3971	6	130	Normal	BM

* Hb denotes haemoglobin, ANC absolute neutrophil count, Plts platelets, TG triglycerides, ALT alanine aminotransferase, CSF cerebrospinal fluid, BM bone marrow, LN lymph node, and NA not available

TABLE 3. Treatment and outcome

Case No.	Treatment*	Time from onset of fever to initiation of treatment (days)	Response‡ (2 months after onset of initial therapy)	Outcome (time from initiation of treatment/ duration of follow-up)
1	VP-16, AraC, DNR	25	No response	Died (20 days)
2	None	60†	None	Died (30 days from diagnosis)
3	VP-16, Methylpred	21	No response	Died (23 days)
4	VP-16, Dexa, CSA	28	Complete response	Relapsed (5 months); reinduced with VP-16, Dexa; died (7 months)
5	VP-16, Dexa	12	Complete response	Relapsed (41 months); reinduced with VP-16, Dexa; still on treatment (49 months)
6	VP-16, Dexa, CSA	15	Complete response	Alive (53 months)
7	None	10†	Spontaneous remission	Alive (14 months)

* VP-16 denotes etoposide, AraC cytarabine, DNR daunorubicin, Methylpred methylprednisolone, Dexa dexamethasone, and CSA cyclosporin A

† Time from onset of fever to diagnosis

‡ Response: resolution of symptoms and normalisation of laboratory results

cyclosporin A. All three had responded at 2 weeks and achieved remission at 8 weeks. Two had subsequent recurrences and one died during the recurrence; the survivor is still undergoing treatment. None of our patients underwent stem cell transplants. Four of our seven patients died, giving an overall mortality of 57%. All deaths were attributed to active HLH disease although one patient's clinical course was complicated by an opportunistic *Candida krusei* fungaemia. All died of coagulopathy and multi-organ failure. Before 1995, the mortality rate was 100%. This was reduced to 25% after the introduction of the HLH-94 treatment protocol (Table 3).

Discussion

Haemophagocytic lymphohistiocytosis is a rare disease—we have seen only seven cases in the past 15 years. The incidence of familial HLH is estimated at 0.12 per 100 000 children in Sweden and the United Kingdom. There are no published incidence rates for secondary HLH but the overall HLH rate would be higher if secondary HLH is included.[3] The observed overall incidence has been increasing due to improvements in diagnosis and case detection.[4] Because effective treatment is now available and early diagnosis and treatment can improve survival, this rare disease deserves greater attention.

Clinical features

Our series demonstrates that the symptoms of HLH can be highly variable and non-specific. Although all of our patients had prolonged fever, massive hepatosplenomegaly and cytopaenia, many also presented with jaundice, liver dysfunction, lymphadenopathy and neurological problems—all symptoms seen in severe hepatitis, sepsis, and malignancies. The non-specific nature of the symptoms leads to difficulty making the diagnosis as shown by the relatively long lag time between disease onset and diagnosis in our series. Many of our patients were initially diagnosed with sepsis or hepatitis and most were extensively investigated for pyrexia of unknown origin and treated for sepsis before the correct diagnoses were made. One patient died before treatment could be initiated. Diagnostic guidelines for HLH based on clinical features, laboratory and histopathological investigations were devised by the Histiocyte Society in 1991 and were

updated in 2004. Most of our patients fulfilled the diagnostic criteria on presentation but HLH is known to follow an atypical course and some patients may not have fulfilled the diagnostic criteria on presentation. Treatment should not be unduly delayed and may need to be commenced on strong clinical suspicion of HLH.[3,5]

Investigations

When the HLH diagnostic guidelines were revised in 2004, three additional diagnostic criteria—ferritin of above 500 μg/L, low or absent NK-cell activity, and soluble CD25 of above 2400 U/mL—were included. We found that serum ferritin level was an important diagnostic feature in patients with prolonged fever and organomegaly. Ferritin levels were grossly raised (range, 6003-15 120 μg/L) in all those patients in whom the ferritin levels were checked. Infections may cause ferritin levels to rise, but the level rarely exceeds 200 μg/L.[6] A ferritin level above 500 μg/L is 84% sensitive for HLH.[3] Therefore, in a child with persistent fever, a ferritin level above 500 μg/L is highly suggestive of HLH. As for NK-cell activity and soluble CD25 levels, these investigations are not readily available in Hong Kong.

Haemophagocytosis was present in the first bone marrow examinations of all our patients, which helped make the diagnosis more obvious. The yield was much higher than that reported in published series where only 32% of patients had initial evidence of haemophagocytosis (85% had haemophagocytosis on later bone marrow examinations).[7] This might be related to the relatively longer time lag from disease onset to the first bone marrow examination in our series. Bone marrow examination should be performed in patients with prolonged fever and organomegaly to exclude malignancies. If the bone marrow shows evidence of haemophagocytosis, this points to the diagnosis of HLH. If it is absent, however, this does not exclude the diagnosis of HLH, and a further bone marrow examination is necessary.[4]

Pathogenesis

Haemophagocytic lymphohistiocytosis is divided into two types, primary and secondary HLH. Primary HLH includes familial HLH and immune deficiency syndromes. Familial HLH is an autosomal recessive disease, and may thus be revealed by a close examination of family histories. Around 70 to 80% of patients with familial HLH present in infancy, but late onset has also been reported.[7] Immune deficiency syndromes including Chediak-Higashi syndrome, Griscelli syndrome, and X-linked lymphoproliferative syndromes are associated with familial HLH. Genetic mutations related to impaired NK cell functioning have been identified in this group of patients. Thus far, at least four types of genetic mutations have been identified in familial HLH.[8] Primary HLH is invariably fatal if untreated, with a median survival of only 2 months.[3] Secondary HLH develops as a consequence of powerful immunological activation of the mononuclear phagocyte system.[3] Severe infections, especially EBV infection, are the commonest infection triggering HLH, particularly in Asia. In Japan, up to 18% of cases of paediatric HLH are associated with EBV.[7] Other viral infections, including those caused by cytomegaloviruses, adenoviruses, B19 parvoviruses, herpesviruses, and Coxsackie viruses have reportedly triggered secondary HLH.[9] A recent report demonstrated that patients with influenza A H5N1 infections have clinical features and postmortem findings remarkably similar to those of patients with HLH.[10] Bacterial and protozoal infections have also been associated with secondary HLH.[7,9] As infections may also trigger primary HLH, acute infection is not a feature that can be used to discriminate between the two types.[4] Secondary HLH is also aggressive, with a mortality rate of 50%.[8]

Connective tissue diseases have also been associated with an HLH-like picture. Patients with macrophage activation syndrome (MAS), an aggressive, fatal condition seen in patients with connective tissue diseases share the same clinical and investigative features including high ferritin, high triglycerides, and defective NK cell functioning, as patients with HLH.[11] Although controversies still exist, some would regard MAS as a form of HLH.[7]

None of our patients were classified as having primary HLH but we were limited by the unavailability of molecular diagnostic testing. Natural-killer cell functioning and molecular gene studies are not readily available in Hong Kong. Gene studies for perforin gene mutation (*PRF1*) were done in Japan for two of our patients and both were negative but a recent collaborative study found that the perforin gene defect is not seen in Asian countries apart from Japan.[12] None of our patients had positive family histories but the family history is often negative because the disease is autosomal recessive.[13] Two of our seven patients had EBV-associated HLH, an incidence comparable to that in Japan.

The pathogenesis of HLH involves an increased inflammatory response and defective cytotoxic function. Excessive stimulation of T-lymphocytes and histiocytes leads to secretion of high levels of cytokines such as interferon-γ, tumour necrosis factor–α, soluble interleukin-2 receptor, interleukin-1 and 6. The hypersecretion of these pro-inflammatory cytokines accounts for the clinical manifestation of high fever, cytopaenia, tissue damage, and organ failure. Despite the excessive activation of cytotoxic cells, the cytotoxic functioning of NK cells and cytotoxic T-lymphocytes is impaired in HLH. Natural-killer cells and cytotoxic T-lymphocytes execute

their killing function through the release of cytolytic granules containing perforin. Perforin is a protein that inserts into the plasma membrane of the target cells, forms pores and induces apoptosis. A defective perforin gene is one of the most studied elements of the pathogenesis of HLH.[4,14] The currently identified genetic defects associated with HLH are related to either perforin function or the cell signaling processes involved in the priming, docking, or releasing of cytolytic granules.[8] Overall, HLH is a result of a vicious cycle of defective cytotoxic cells failing to remove the antigen or infected cells that in turn persistently stimulate NK cells and cytotoxic T-lymphocytes. These secrete high levels of pro-inflammatory cytokines, causing sustained activation and clonal proliferation of cytotoxic cells and organ damage.

Treatment

Effective treatment exists. In 1994, the HLH Study Group of the Histiocyte Society designed a treatment protocol using VP-16, dexamethasone, and cyclosporin.[13] Initial treatment aims to suppress the hyperinflammatory response and the cytokines released by activated lymphocytes and histiocytes, which cause the life-threatening symptoms. The secondary aim is to eradicate the stimulus for ongoing but ineffective NK cell and T-suppressor cell activity.[7]

Etoposide is a cytotoxic drug highly effective in monocytic and histiocytic diseases. It is an excellent initiator of apoptosis. It has also been shown to inhibit synthesis of EBV nuclear antigen and can therefore prevent expansion of EBV-infected T cells.[7] Secondary acute myeloid leukaemia associated with VP-16 has been reported, but was not observed in our series. Evidence from previous series shows that the risk of acute myeloid leukaemia is small and does not justify withholding VP-16 in children with HLH.[13,15] Corticosteroids are cytotoxic for lymphocytes, inhibit dendritic cell differentiation, and suppress the expression of cytokines. Dexamethasone is the drug of choice due to its high penetration into the central nervous system. Cyclosporin is an immunosuppressive drug shown to be effective in HLH. In the revised treatment protocol issued in 2004, the introduction of cyclosporin was advanced from the eighth week to the commencement of treatment. A review of the 10-year outcome of the HLH-94 protocol showed that HLH activity was most aggressive during the initial 2 months, hence there was a need to escalate treatment intensity in the first 2 months without increasing myelotoxicity. The HLH protocol essentially targeted primary HLH, however, as it is often impossible to differentiate between primary and secondary HLH, treatment is recommended in all patients with severe or persistent symptoms. As for primary or persistent HLH, the ultimate curative treatment is a stem cell transplant.

Prognosis

The high mortality rate (57%) in our series illustrates the aggressive nature of HLH. Only two patients survived without major sequelae. Primary HLH is invariably fatal if untreated. Secondary HLH is also aggressive with a quoted mortality of 50%. Reports from Japan and other countries indicate that EBV-associated HLH can have fatality rates as high as 40%.[16] With the introduction of a standard treatment protocol in 1994, the 5-year survival of HLH has improved markedly, from 5-22% to 55%.[3] Prompt initiation of treatment is important. The 100% mortality early in our series was related to the relatively long lag time from disease onset to initiation of treatment. Our series clearly shows that prompt initiation of treatment and use of the treatment protocol improves survival.

Conclusion

Haemophagocytic lymphohistiocytosis is a rare but highly fatal disease if not diagnosed and treated early. The diagnosis should be considered in children with persistent fever, organomegaly, and cytopaenia. Elevated serum ferritin and the presence of haemophagocytosis in bone marrow samples may help to establish the diagnosis. Diagnostic guidelines and effective treatment are available. Prompt diagnosis and initiation of treatment can improve prognosis, thus there is a need for heightened awareness of this disease entity.

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