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# **Evaluation of Children with Haemophagocytic Lymphohistiocytosis (HLH) at Red Cross War Memorial Children's Hospital 1991-2010**

**by**

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In fulfilment of the requirements for the degree

MMed in Paediatrics

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

Date of submission :15 June 2011

Supervisor: Dr Marc Hendricks

Red Cross War Memorial Children's Hospital Paediatric Haematology Oncology Service

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DECLARATION

I, ...*Juli Switala*....., hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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## 1. List of Abbreviations

ADF	Alive disease free
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
ATG	Anti-thymocyte globulin
AWD	Alive with disease
BM	Bone marrow
CMV	Cytomegalovirus
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computed tomography
DD	Died of disease
DIC	Disseminated intravascular clotting
DU	Death unrelated
EBV	Epstein Barr virus
ESR	Erythrocyte sedimentation rate
FHLH	Familial HLH
Hb	Haemoglobin
HDL	High density lipids
HIV	Human immunodeficiency virus

HLH	Haemophagocytic lymphohistiocytosis
IAHS	Infection related HLH
IL	Interleukin
IFN	Interferon
IVIG	Intravenous immune globulin
JRA	Juvenile rheumatoid arthritis
LB	Liver biopsy
LDH	Lactate dehydrogenase
LDL	Low density lipids
LN	Lymph node
LP	Lumbar puncture
LPI	Lysinuric protein intolerance
MAHS	Malignancy related HLH
MAS	Macrophage activation syndrome
NCC	No clear cause
NKC	Natural killer cells
NSAID	Non-steroidal anti-inflammatory drug
Plts	Platelets
RSV	Respiratory syncytial virus
SLE	Systemic lupus erythematosus

TBM	Tuberculous meningitis
TG	Triglyceride
TNF	Tumour necrosis factor
VL	Viral load
VLDL	Very low density lipids
WBC	White blood count
XLP	X- linked lymphoproliferative syndrome

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## 2. Letters of Intent

11 May 2010

School of Child and Adolescent Health

Dear Professor Zar

Re: Research Proposal for Ethics Committee approval.

Please find attached my submission for your approval, for a research project I wish to commence as part of my MMed during 2010. I plan to review the folders of patients admitted at Red Cross Children's Hospital diagnosed with Haemophagocytic Lymphohistiocytosis. My supervisor for this study is Dr Marc Hendricks.

Kind Regards

Juli Switala

Paediatric Registrar- Red Cross Children's Hospital

University of Cape Town

11 May 2010

Ethics Committee  
University of Cape Town  
Dear Colleague

Herewith I attach my application for ethics approval for my study 'Evaluation of Children with Haemophagocytic Lymphohistiocytosis (HLH) at Red Cross Children's Hospital'. This is a descriptive retrospective study of patients who have been treated for this disease at Red Cross Children's Hospital. I have received scientific approval from the School of Child and Adolescent Health and I would like to request an expedited approval as my study is a retrospective folder review, requiring no further recall of patients and no further investigations.

This is a study that I am doing as part of my MMed and my supervisor is Dr Marc Hendricks at Red Cross Children's Hospital.

Kind Regards,

Juli Switala

Paediatric Registrar –Red Cross Children's Hospital

## 1. Research Protocol

### **Evaluation of Children with Haemophagocytic Lymphohistiocytosis (HLH) at Red Cross War Memorial Children's Hospital**

#### **Background**

Haemophagocytic Lymphohistiocytosis (HLH) is a rare haematological disorder in children. An estimated incidence of 0.12 per 100 000 children per year was found in a Swedish (Henter, Stockholm) study<sup>1</sup>. However, this is probably an underestimation due to the difficulty in diagnosing the disease.

HLH is characterized clinically by persistent fevers, organomegaly, cytopaenias and typical biochemical derangements viz. hypertriglyceridaemia, hyperferritinaemia and hypofibrinogenaemia. Other associated findings include decreased natural killer cell (NKC) function and raised soluble CD 25.

The exact pathophysiology of HLH is not completely understood but involves a trigger (often an infection) which sets off an uncontrolled inflammatory cascade, characterized by an increase in hyperactivated macrophages and T lymphocytes which leads to increased production of cytokines, alongside reduced cellular cytotoxicity as a result of reduced or absent NKC function. When functioning normally, NKCs play a role in lysis of cells by releasing cytotoxic proteins - perforins and granzymes, as well as by release of soluble mediators IFN-gamma, GM-CSF and TNF in response to pathogens. Once the foreign protein is destroyed, the stimulus for the immune activation is removed, and the reaction subsides. It follows therefore, that poor NKC function allows foreign proteins to persist and disseminate, causing increased, sustained activation of macrophages and a disproportionate increase in CD8 cells. This uncontrolled inflammatory response causes macrophage infiltration of tissue, as well as pro-inflammatory cytokine release, which likely accounts for the tissue damage and clinical picture associated with the disease<sup>2,3</sup>. TNF may be the cause of the hypofibrinogenaemia (it acts as a procoagulant) and the hypertriglyceridaemia<sup>3</sup>.

HLH is induced by strong, dysfunctional immunological activation. In primary HLH this arises as a result of a variety of mutations of the genes which encode for proteins involved different aspects of the granule dependant cytotoxic function of the NK cell, such as perforin production<sup>3</sup>. Predominant mutations vary between different ethnic groups<sup>3</sup>. In secondary HLH this dysfunction may be related to infection, malignancy or auto-immune disease.

HLH secondary to infection may be misdiagnosed as an ordinary infection<sup>4</sup> due to the similar clinical syndrome<sup>1</sup>. Infection may also be linked to both onset and recurrences of primary HLH<sup>5</sup>. Epstein Barr Virus is a common trigger for both conditions<sup>5</sup>, and also carries the worst prognosis of the infection-associated HLH subtypes<sup>6</sup>. Other viruses (especially those in the herpes group), bacteria, protozoa, fungi and rickettsiae may also lead to the condition<sup>6</sup>.

In addition, HLH may herald the onset of malignancies or auto-immune disease. It has been described in ALL, germ cell tumours, thymomas and carcinomas. It may present with a masked haematolymphoid malignancy such as lymphomas<sup>6</sup>.

Auto-immune related HLH, more commonly known to rheumatologists as macrophage activation syndrome, can occur as a result of rheumatoid arthritis and SLE.

The trigger in auto-immune related HLH may be viral infection or drugs, such as methotrexate or NSAIDs<sup>7</sup>.

On reviewing 122 children on the international registry Arico *et al* found a 5 year survival of 10% in children receiving chemotherapy alone and 66% for those who received an allogenic bone marrow transplant<sup>8</sup> with a median survival time of 6 months in untreated children<sup>2</sup>. The disease is uniformly fatal in the absence of an HLA identical sibling bone marrow transplant, although chemotherapy alone may prolong survival<sup>1</sup>. Death may be due to neutropaenic sepsis, multi-organ failure or CNS manifestations<sup>4</sup>.

The early diagnosis and initiation of appropriate treatment in HLH is crucial as it arrests the cytokinaemia and induces remission. In secondary phenomena, the diagnosis and control of precipitating disease entities is crucial in arresting evolving clinical disease and preventing death.

Although the importance of timely diagnosis of HLH has been highlighted, it may be a difficult diagnosis to make. The diagnosis is one of exclusion, and is typically made in a child with a documented, unremitting fever- often without a clear cause, with associated cytopenias and organomegaly.

Diagnostic criteria were established by the Histiocyte Society in 1991 and include fever, splenomegaly, cytopenias involving at least two lineages, hypertriglyceridaemia and/or hypofibrinogenaemia and haemophagocytosis in bone marrow, spleen or lymph nodes. Five of these 8 features or a molecular diagnosis of HLH is needed to make the diagnosis<sup>5, 9</sup>. Criteria were adjusted in 2004 to include low NK activity, hyperferritinaemia and raised serum concentrations of IL-2 receptor- $\alpha$  (CD 25). The alpha chain of IL-2 receptor increases during active disease making it a good marker<sup>4</sup>. This occurs as a result of increased number of activated lymphocytes.

This lymphocytosis also explains the hepatosplenomegaly (with transaminitis and hyperbilirubinaemia) and the many manifestations of CNS disease<sup>4</sup>. Although serum CD 25 may differentiate HLH from other conditions, the time delay, and lack of availability at many centres limit its usefulness<sup>9</sup>. Raised serum ferritin and decreased serum fibrinogen occur as a result of activated macrophages which secrete ferritin and promote high levels of fibrinolysis<sup>4</sup>.

Allen *et al* have shown a ferritin level  $>10\,000$  mcg/l to be 90% sensitive and 96% specific for HLH<sup>9</sup>. Hyperferritinaemia is not unique to HLH and other differentials must be considered. Lysinuric protein intolerance (LPI), is an autosomal recessive disease in which high ferritin levels are present<sup>10</sup>. Congenital haemochromatosis also causes hyperferritinaemia and may mimic neonatal HLH, as both can present with deranged liver functions, similar clinical phenotypes and hepatomegaly<sup>10</sup>. Hereditary Hyperferritinaemia-Cataract Syndrome is another rare cause of hyperferritinaemia<sup>10</sup>.

Raised serum triglycerides are found early in the disease, and resolves with remission. LDL and VLDL levels are usually markedly increased, and HDL decreased<sup>1</sup>.

Because of the potential morbidity and mortality encountered in this disease, early diagnosis is paramount. We will review the epidemiological data of these patients including their biochemical markers in an attempt to gauge their usefulness as early indicators of HLH.

## **Objectives**

1. To evaluate the epidemiological features of children with HLH.
2. To evaluate different biochemical markers of disease and to assess their usefulness as predictors of disease.
3. To assess whether threshold values for ferritin and triglycerides are helpful in identifying those who are most at risk / likely of having HLH.
4. To evaluate time to diagnosis and treatment in our study group compared to international norms.

## **Methodology**

### **1.Design**

A retrospective folder review.

### **2.Sample**

15 patients treated at Red Cross War Memorial Children's Hospital

### **Inclusion criteria:**

Any child with confirmed HLH as assessed by 2004 HLH diagnostic criteria<sup>4</sup>.

### **Exclusion criteria:**

None.

### **3. Data**

Patient notes will be reviewed and a database will be created.

### **4. Analysis**

Data will be descriptively reported with specific reference to the questions set out in the objectives.

### **5. Dissemination Plan**

The study results will be prepared for submission for an MMed thesis. Findings of the study will be presented at Research Day as a vehicle to educate local staff about early diagnosis and treatment. We will submit our findings for publication to the Journal of Paediatric Haemataology and Oncology or an equivalent.

### **6. Costs**

There will be no costs involved in this study.

### **7. Conflict of Interests**

The authors wish to declare no conflict of interest.

### **8. Ethical Considerations**

As this is a retrospective folder review, patient management will not be affected in any way. Complete confidentiality will be ensured as patients will have numeric identifiers. No further recall of patient will be necessary and no further investigations will be undertaken. Patients will not incur any expense or inconvenience as a result of the review.

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## Data Collection Sheet

Folder number

Date of Birth

Sex

Race

Date of admission

Date of diagnosis

Age at diagnosis

Precipitants

Family History

### Biochemical markers

- Ferritin
- Triglycerides
- Fibrinogen
- Haemoglobin
- White cell count
- Platelets
- EBV Viral load
- LP result
- Bone Marrow to confirm results

## Clinical

- Fever and duration
- Hepatomegaly
- Splenomegaly
- CNS features
- Other clinical features
- Infections

## Treatment

- Drugs
- Duration
- Remission

Principal Investigator

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## 2. Ethics Approval



UNIVERSITY OF CAPE TOWN

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Research Ethics Committee  
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17 June 2010

HREC REF: 283/2010

Dr J Switala  
Paediatrics  
Red Cross

Dear Dr Switala

**PROJECT TITLE: EVALUATION OF CHILDREN WITH HAEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS (HLH) AT RED CROSS CHILDREN'S HOSPITAL**

Thank you for your ethics submission to the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the FHS HREC has **formally approved** the above-mentioned study.

**Approval is granted for one year until 30 June 2011.**

Please send us an annual progress report if your research continues beyond the approval period. Alternatively, please send us a brief summary of your findings so that we can close the research file.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

**Please quote the REC REF in all your correspondence.**

Yours sincerely

**PROFESSOR M BLOCKMAN**  
**CHAIRPERSON, HSF HUMAN ETHICS**

Federal Wide Assurance Number: FWA000011637.  
Institutional Review Board (IRB) number: IRB00001938

tmjell

### **3. Literature review**

#### **Evaluation of Children with Haemophagocytic Lymphohistiocytosis (HLH) at Red Cross Children's Hospital.**

##### **Background**

Haemophagocytic Lymphohistiocytosis (HLH) is a rare haematological disorder in children. An estimated incidence of 0.12 cases per 100 000 children per year was reported in a Swedish study<sup>1</sup>. However, this is probably an underestimation due to the difficulty in diagnosing the disease.

HLH is characterised clinically by persistent fevers, organomegaly, cytopaenias and typical biochemical derangements - primarily hypertriglyceridaemia, hyperferritinaemia and hypofibrinogenaemia. In 1952 Farquhar and Claireaux described what is now known to be familial HLH<sup>2</sup> and in 1979 Risdall described similar findings in immunosuppressed patients<sup>3</sup>. These patients often had proven herpes or adenovirus infection and all had bone marrow and lymph node infiltration, lymphocyte depletion, hepatocellular necrosis and leptomeningeal involvement<sup>4</sup>.

##### **Pathophysiology**

The exact pathophysiology of HLH is not completely understood. It is thought to arise as a result of a hyperactivated, yet inefficient immune system. When functioning normally, NKCs play a role in lysis of cells by releasing cytotoxic proteins - perforins and granzymes, as well as by release of soluble mediators IFN-gamma, GM-CSF and TNF-alpha, IL1, 6, and 10 and macrophage colony stimulating factor in response to pathogens<sup>5</sup>. Once the foreign protein is destroyed, the stimulus for the immune activation is removed. In HLH, foreign cells stimulate T-cell activation and proliferation, but then fail to remove the stimulus due to poor NKC function, allowing foreign material to persist and disseminate, activating more T-cells and macrophages, worsening the cytokinaemia and causing end organ damage<sup>6</sup> and a disproportionate increase in

CD8 cells. This uncontrolled inflammatory cascade and organ infiltration likely accounts for the tissue damage and clinical picture associated with the disease<sup>6,7</sup>.

The trigger for this cascade is typically a viral infection, but may be any infection, a toxin, a metabolic product, profound stress or tissue damage<sup>8</sup>. Chemotherapy and immune suppression post transplantation may also activate the immune system<sup>8</sup>, although the exact mechanism is not well delineated.

### **Classification**

HLH is not a single disease but a clinical syndrome, induced by strong, dysfunctional immunological activation. Primary and secondary forms of the disease are histologically indistinguishable.

#### **A: Primary HLH**

The immune defect may be genetic (primary HLH), in which the actual mechanism of NK cell dysfunction, viz. poor perforin or granzyme production, or poor exocytosis of granules, is a function of the gene mutation. HLH is induced by strong, dysfunctional immunological activation. In primary HLH this arises as a result of a variety of possible mutations of the genes which encode for proteins involved in different aspects of the granule dependent cytotoxic function of the NK cell, affecting processes such as perforin or granzyme production, or exocytosis of granules<sup>7</sup>. Predominant mutations vary between different ethnic groups<sup>7</sup>.

The inheritance may be autosomal recessive, with a reported frequency of one per 50 000 births<sup>5,9</sup>. Seventy percent of patients develop symptoms in the first year of life<sup>1,5</sup>. *In utero* development of primary HLH has been described and the upper age limit is not known<sup>1</sup>.

Different gene mutations result in different pathophysiological mechanisms of disease by affecting either the NK cell or perforin production. The exact gene mutations differ in patients with different ethnicities<sup>6,10</sup>. The perforin gene PRF1 mutations are currently thought to be the most common genetic defects in HLH<sup>6</sup>, but perforin mutations only occur at a frequency of

between 20-30%<sup>8</sup>. Other implicated genes include 10q21–22, UNC13D and STX11<sup>10,11</sup>. In a study by Lee *et al*, British and American patients of African descent were all shown to have the same gene mutation on the perforin gene PRF1 (50delT) which occurred exclusively in this ethnic group<sup>12</sup>. Similarly, Celtica *et al* found that 90% of patients of African descent had this founder mutation<sup>10</sup>. Phenotypically, it is associated with an earlier age of onset compared to other PRF1 mutations. By comparison, the most common mutation amongst Turkish patients (*p.W374X*) is not exclusive to this group, possibly as a result of gene migration<sup>10</sup>.

Two subgroups of primary HLH are described:

- 1) Familial HLH (FHLH), a group in which HLH is the only manifestation.
- 2) A second group, in which HLH is a sporadic but frequent association, but not the only feature of disease. This is found in children with primary immune deficiencies like X-linked lymphoproliferative disease, Chediak Higashi syndrome or Griscelli syndrome<sup>5,13</sup>.

## **B: Secondary HLH**

Secondary HLH is induced by an immunological stressor, typically an infection, but malignancy and auto-immune disease may also precipitate the syndrome. A trigger is not always identified, making differentiation between primary and secondary HLH difficult. There is no way to reliably differentiate primary from secondary disease clinically, although secondary HLH tends to present after one year of age<sup>8</sup>.

### **1) Infection related HLH (IAHS)**

Secondary HLH is typically related to infection. The relationship between infection and HLH is a complex one. Due to the similarity of the clinical features, HLH may be misdiagnosed as an infection initially. When both occur concurrently the infection may be the trigger of IAHS or primary HLH<sup>1, 4, 11, 13</sup>, or be a result of the decreased immunity associated with HLH.

Epstein Barr Virus (EBV) is a common trigger for both primary and secondary HLH<sup>11</sup> and carries the worst prognosis of the IAHS groups<sup>4</sup>. EBV is reported to have been isolated in 74% of viral-

associated cases<sup>5</sup>. It has a higher prevalence in Asia than in western countries and this may be due to genetic or environmental factors<sup>8</sup>. The spectrum of infectious precipitants of HLH is substantial. Viruses, especially those in the herpes group, bacteria, protozoa, fungi and rickettsiae have all been implicated<sup>4,10</sup>. HIV, *M.Tuberculosis* and *Plasmodium spp.* have also been reported<sup>14</sup> - all important diseases in sub-Saharan Africa. IAHS has a reported mortality rate of more than 50% in children by Janka *et al*<sup>8</sup>.

## **2) Malignancy associated HLH (MAHS)**

The incidence of MAHS is not known. Two patterns have been described<sup>4</sup>.

1) The first is characterised by HLH which develops as a result of an infection or unknown trigger, long before or during treatment for malignancy. The malignancy itself may respond to treatment and HLH may only become evident months later. Malignancies reported to display this pattern of presentation include acute lymphoblastic leukaemia, multiple myeloma, germ cell tumours, thymomas and certain carcinomas.

2) The second is characterised by HLH which develops rapidly in an apparently healthy child or adult, in which the malignancy is initially masked but later diagnosed. This has been reported preceding T- cell leukaemia, anaplastic large cell lymphoma, and adult B-cell lymphoma.

## **3) Auto-immune related HLH**

Also known as macrophage activation syndrome (MAS), it was first described in 1985 in patients with rheumatoid arthritis and SLE<sup>8</sup>. Up to 7% of patients with systemic juvenile rheumatoid arthritis may develop MAS with a reported mortality rate of 10 -20%<sup>13</sup>. Triggers include viral infections or drugs such as methotrexate or NSAIDs<sup>5</sup>.

## **Diagnosis**

### **Introduction**

HLH may be extraordinarily difficult to diagnose. The importance of early detection cannot be overstated, as the commencement of medical therapy is critical to survival. The diagnosis is one of exclusion and is typically made in a child with a documented, unremitting fever, often without a clear cause, with associated cytopaenias and organomegaly. A high index of suspicion is required, in a clinical setting where the differential diagnosis may be broad and the clinical course may vary considerably. Atypical presentations may further complicate matters. For example, neonatal onset disease may have a less prominent fever, which is usually a cardinal feature<sup>1</sup>. In addition, some patients experience improvements in their symptoms following non-specific therapies like blood transfusions or antibiotics<sup>8</sup>. The average time to diagnosis varies between studies from seven days to four weeks<sup>15, 16</sup>.

In 1991 the Histiocyte Society proposed a set of diagnostic criteria which included documented fever, splenomegaly, and cytopaenias involving at least 2 lineages, hypertriglyceridaemia and/or hypofibrinogenaemia and haemophagocytosis in bone marrow, spleen or lymph nodes. The criteria were amended in 2004 to include low NK activity, hyperferritinaemia and raised levels of serum IL-2 receptors (CD25). Five of these eight features or a molecular diagnosis of HLH is needed to make the diagnosis (Table I)<sup>11</sup>. Since then a slight modification has been proposed, but not yet codified (Table II). These modifications incorporate more clinical features, are less stringent and allow for the diagnosis of 'suspected HLH'.

### **Clinical criteria and features**

70-80% of patients with FHLH are symptomatic within the first 12 months of life<sup>13</sup>. Secondary HLH typically presents later in life, but both diseases ultimately run a rapidly fatal course<sup>16</sup>, unless treatment is instituted. Each clinical feature and biochemical abnormality is as a result of either the excessive cytokine release or organ infiltration by activated lymphocytes.

## **Fever and Tissue Involvement**

Fever is caused by the release of IL-1 and IL-6, and is typically protracted<sup>13</sup>. Henter *et al* compared the frequency of initial clinical features in patients on the FHL registry with those in a population based study, as well as those found in a literature review. In all three populations fever and splenomegaly were present in more than 90% of patients. Similarly, hepatomegaly was present in 94% of the patients in the literature review and 97% of the study patients, but insufficient data was available for the registry population. Hepatosplenomegaly is reported to be marked and progressive and is accompanied by transaminitis and hyperbilirubinaemia<sup>1</sup>. Lymphadenopathy and skin rash were less consistent findings, varying from 17-51% and 6-65% respectively between the groups<sup>1</sup>. The rash is usually transient and associated with fever<sup>1</sup>. Pulmonary symptoms may also develop as a result of infiltration by activated lymphocytes.

## **Neurological disease**

Neurological features associated with HLH include seizures, hyper- or hypotonia, a bulging fontanel, irritability, neck stiffness, ataxia, blindness, encephalopathy, psychological depression, opisthotonus, hemi- or tetraplegia, a depressed level of consciousness and 6<sup>th</sup> and 7<sup>th</sup> cranial nerve palsies<sup>1, 13, 16</sup>. These may be present early in the disease or may evolve later<sup>1, 16</sup>. Haddad *et al* found that 25 of 34 patients had CNS involvement at presentation. Twenty of these 25 had CSF abnormalities with no clinical features apart from neck stiffness<sup>17</sup>. In this study, development of CNS symptoms was associated with an inferior outcome. Seizures were found to be the commonest manifestation in young patients. In a series by Hallahan *et al* 75% of patients developed CNS involvement at some point during the course of disease, but most did not present with features initially. Again, CNS involvement worsened prognosis in this group<sup>16</sup>.

## **Biochemical markers and investigations**

Typical serum biochemical findings include hypertriglyceridaemia, hypofibrinogenaemia or coagulopathy, elevated LDH, liver dysfunction and marked transaminitis, hyperferritinaemia, raised VLDL and decreased HDL and raised cytokines in both serum and CSF<sup>9</sup>. These changes may be present initially or develop later, usually normalising after successful treatment<sup>8</sup>.

## Ferritin

Ferritin is an iron binding protein consisting of light and heavy chains. Oxidative stress and pro-inflammatory cytokines from the NKC pathway up-regulate transcription of the ferritin heavy chains. Ferritin can therefore be used as an indirect measure of cytokine activation<sup>15</sup>.

As a ferritin level may be acquired much faster than a functional NKC assay or IL-2  $\alpha$  receptor in most centres, it is of more practical significance in the acute setting<sup>15</sup>.

Hyperferritinaemia is not unique to HLH and other differentials must be considered. Lysinuric protein intolerance (LPI) is an autosomal recessive disease in which high ferritin levels have been reported. This is now understood to be due to a form of HLH related to LPI<sup>18</sup>. Congenital haemochromatosis also causes hyperferritinaemia and can mimic neonatal HLH, since both can present with deranged liver function, a similar clinical syndrome and hepatomegaly<sup>18</sup>. Hereditary hyperferritinaemia-cataract syndrome is another rare cause of hyperferritinaemia<sup>18</sup>. Ferritin may be elevated in infections but values are not likely to exceed 200mcg/l<sup>8</sup>. In a study by Allen *et al*, a ferritin level >10 000 mcg/l was found to be 90% sensitive and 96% specific for HLH (98% if fever was added as a criterion)<sup>15</sup>. By comparison serum ferritin levels in JRA without MAS may exceed 10000mcg/l<sup>8</sup>. In a series of patients including both viral- and malignancy associated HLH, Esumi *et al* found ferritin levels to be elevated at diagnosis, with further increases at the onset of DIC. Patients with sustained high ferritin levels after three months died, while those with decreased levels survived, suggesting that ferritin level may be a useful marker of disease activity and not just the presence of disease<sup>19</sup>.

## CD 25 (IL 2 $\alpha$ receptors)

By comparison the alpha chain of the IL-2 receptor is made by activated histiocytes and T-cells. It increases during active disease and is cell specific. Janka *et al*<sup>8</sup> found it to be the most sensitive marker (93%) for HLH. Specificity is also high<sup>8,15</sup> making it a good marker to differentiate HLH from infection. Diagnosis may be complicated by the fact that elevated levels may also be found in leukaemias and lymphomas<sup>8</sup>. The lack of availability to perform this test at many centres and the time required to obtain results limits its usefulness<sup>15</sup>.

## **Fibrinogen**

Hypofibrinogenaemia may not be present on initial testing but may develop later. Depressed serum fibrinogen levels occur as a result of the increased numbers of activated macrophages secreting plasminogen activating factor, which promotes high levels of fibrinolysis<sup>13</sup>. Risdall *et al* reported clotting abnormalities in 68% of patients with viral-associated HLH<sup>3</sup>. These consisted of prolonged partial thromboplastin and thrombin times and normal to prolonged prothrombin times<sup>3</sup>.

Hypofibrinogenaemia may be useful in differentiating HLH from infection, as in most infections (other than DIC), fibrinogen is typically normal or raised<sup>8</sup>. Janka *et al* reported a sensitivity of only 53%<sup>8</sup>.

## **The Bone Marrow**

Pancytopenia is ascribed to the effects of TNF-  $\alpha$  and interferon-gamma on bone marrow precursor cells<sup>13</sup>. The platelet count typically increases during remission and may decrease at relapse. These changes occur relatively early on, making the platelet count a useful monitor for active disease<sup>1</sup>. The reticulocyte count may be inappropriately low for the severity of the anaemia, due to the increased, but ineffective erythropoiesis in the bone marrow<sup>8</sup>, not unlike other bone marrow failure syndromes, like aplastic anaemia or leukaemia.

Bone marrow aspiration may provide histological evidence of HLH and help to exclude malignancies. The classic features are histiocyte hyperplasia and widespread infiltration of normal haemophagocytic macrophages and lymphocytes<sup>3,6,8</sup>. The bone marrow appearance varies considerably depending on when in the course of the disease it is performed<sup>6</sup>.

The haemophagocytosis may be absent initially, and repeated aspirations may be required to demonstrate it. When it develops, histiocytes are most commonly seen engulfing erythrocytes but may also phagocytose lymphocytes and platelets<sup>8</sup>. Cellularity may wane in later stages or after therapy, giving the bone marrow an aplastic appearance<sup>1</sup>. Histiocyte hyperplasia and haemophagocytosis may persist for up to 5 months after recovery in some patients, but in others normalises sooner<sup>3</sup>.

## **Triglycerides**

Hypertriglyceridaemia is usually found early in the disease, and resolves as the disease remits<sup>1</sup>. This occurs as a result of the inhibition of lipoprotein lipase by TNF- $\alpha$ <sup>13</sup>. LDL and VLDL levels are usually markedly increased, and HDL decreased<sup>1</sup>.

## **Elevated LDH**

Imashuku *et al* found a higher incidence of raised LDH than hypofibrinogenaemia and hypertriglyceridaemia, suggesting that it may be a useful marker for active disease<sup>20</sup>.

## **Natural Killer Cells**

NKC dysfunction is a cardinal feature of HLH. In FHLH the number of NKCs may be normal, but defective. This defect persists even between episodes of active disease. In secondary HLH, both the number and function of NKCs are deficient during active disease with function typically, but not always, normalising between episodes<sup>8,13</sup>.

## **Cerebrospinal Fluid and Neuro-Imaging Abnormalities**

Cerebrospinal fluid (CSF) may have raised protein, lymphocytes and occasional erythrocytes<sup>5</sup>. CSF changes may be present even without any clinical features<sup>8</sup> or conversely CSF may appear normal, even in patients with symptomatic CNS involvement<sup>16</sup>. For this reason, Janka *et al* recommend a lumbar puncture be done in all patients<sup>8</sup> as CNS involvement is associated with a worse outcome<sup>16</sup>. CSF neopterin levels may be a useful marker of disease as it indicates histiocyte activity<sup>16</sup>. Neuroimaging may show some features of ongoing or previous inflammation or demyelination. Findings include haemorrhage, infarction, atrophy and oedema<sup>1,16</sup>.

## **Tissue Biopsy**

When indicated and where possible, histological examination of tissue such as nodes, liver, and spleen show widespread infiltration of macrophages and lymphocytes<sup>9</sup>.

## Treatment

Initial management is aimed at arresting the life threatening hyperinflammatory process. Once control of the cytokine response has been achieved, the stimulus for its continuous activation needs to be removed. Finally, as in FHLH, the dysfunctional immune system needs to be permanently replaced<sup>8</sup>. Treatment modalities therefore include chemotherapy, immunotherapy, bone marrow transplant and the treatment of triggers and complications.

Corticosteroids arrest the cytokine cascade by destroying lymphocytes, inhibiting dendritic cell differentiation and reducing chemokine and cytokine expression<sup>8</sup>. Dexamethasone is preferred to prednisolone as it has superior blood brain barrier penetration<sup>11, 13</sup>.

Etoposide is an epipodophyllotoxin derivative. It was first used for treatment of HLH in 1980<sup>8</sup>. It reduces the activation of the cytotoxic cells by destroying the antigen presenting cells<sup>13</sup>. It also inhibits synthesis of EBV nuclear antigen, and may prevent clonal expansion of virus-infected cells<sup>8</sup>. Ambruso reported prolonged remission with its introduction, in combination with steroids in 1980<sup>21</sup>. Etoposide is myelotoxic which may pose a problem in a disease in which cytopaenia and immunodeficiency are prominent<sup>11</sup>. There is also a concern about the risk of developing a secondary leukaemia with the use of etoposide, as acute myeloid leukaemia and myelodysplastic syndrome have been reported in associated with epipodophyllotoxin derivatives<sup>1,9</sup>. This risk is considered to be of less significance than the risk of under treatment for HLH<sup>1</sup>. The risk of developing secondary AML, which is dose related, may be reduced by combining its use with immunotherapy (cyclosporin)<sup>9</sup>. Most modern protocols keep the cumulative etoposide dose well below the threshold which would put one at risk for a secondary leukaemia.

Cyclosporin has been used effectively to induce remission in HLH by affecting T-lymphocyte action and macrophage function. It interferes with the cyclophilin pathway thus causing immunosuppression<sup>8</sup>. It has been used successfully as a single agent for maintenance in HLH, rheumatic disease and MAS<sup>10</sup>. Another immune modulatory agent, antithymocyte globulin (ATG) has been shown to induce remission and may in future be used in resistant cases as part of salvage therapy<sup>1,9</sup>.

Immunoglobulins act by cytokine and pathogen-specific antibodies which stimulate Fc receptor function<sup>8</sup>. Intravenous recombinant immune globulin (IVIG) has been shown to be of use in children with IAHS, in some cases inducing a response even when administered alone<sup>4</sup>.

Ladisch *et al* found that the plasma of three patients with HLH had a lymphoproliferative inhibitor effect on normal, control mononuclear cells<sup>22</sup>. Although the reason for this is not entirely understood, this finding implies that removal of a plasma factor by exchange transfusions potentially reduces this inhibition. However, transfusions during relapses showed less response. These findings may be due to the ability of red cells to absorb cytokines<sup>8, 22</sup>.

Bone marrow transplantation is the only way to restore a normal immune system, and it can be curative in up to 2/3 of patients<sup>8</sup>. Best results are achieved with HLA-matched siblings<sup>11</sup>. If this is not possible a matched unrelated donor is advised<sup>11</sup>.

### **Current Treatment Recommendations**

The Histiocyte Society developed a study group with diagnostic guidelines in 1991 and the first treatment recommendations in 1994. This was known as the HLH-94 protocol. With the collective experience gained from this protocol, HLH-2004 was developed which included revisions to the diagnostic criteria as well as treatment guidelines<sup>11</sup>. Appropriate treatment differs depending on the type of HLH, identification of a trigger and the severity of symptoms. If an infective agent is identified, anti-infective therapy may be the only treatment needed in a mild form, although this is usually not the case<sup>8</sup>.

In mild to moderate secondary HLH, corticosteroids and immunoglobulins may be sufficient<sup>8, 13</sup>. However, mild cases may progress rapidly and even in patients with proven EBV-related HLH, etoposide based regimens have a better outcome than those on corticosteroids, cyclosporin A, immunoglobulins or a combination of these<sup>8</sup>. Finding an infectious cause in children older than 1 year is not sufficient to exclude primary HLH. Janka *et al* advise treating all critically ill children and those less than 1 year according to HLH protocol as the risk of under treating a patient with primary HLH outweighs the risks of over treating a child with secondary HLH. HLH-2004

recommends starting all patients, regardless of evidence of genetic disease, and viral infections on multi-agent intensive therapy<sup>11</sup>.

HLH-94 recommended using a combination of steroids and etoposide, and in some patients, intrathecal methotrexate, as initial therapy for 8 weeks. Continuation therapy included dexamethasone pulsed therapy, etoposide and the introduction of cyclosporin A. This aimed to stabilise patients awaiting bone marrow transplant<sup>9</sup>.

In HLH-2004, initial therapy is intensified by including cyclosporin A from the outset<sup>11</sup>. This modification was made as some patients had died from the disease itself during the first 8 weeks of treatment<sup>11</sup>. In patients who achieve resolution after the initial phase and have no family history, treatment is stopped<sup>11</sup>.

In patients with verified primary HLH or severe, persistent or reactivated secondary HLH, continuation therapy should be continued while awaiting a bone marrow transplant<sup>11</sup>.

Reactivation is part of the clinical picture of primary HLH, and these are most likely as the intensity of therapy is reduced or a new immune response trigger occurs (e.g. viral infections, vaccinations). Intensification of therapy is recommended in these patients<sup>11</sup>.

In patients with CNS involvement, adequate systemic therapy has been shown to decrease active CNS disease<sup>11</sup> and intrathecal therapy alone has not been particularly effective<sup>9</sup>. It is not known whether or not intrathecal therapy in addition to initial systemic therapy would be of added benefit<sup>11</sup>. As such, both HLH-94 and HLH-2004 recommend intrathecal methotrexate be reserved for patients with evidence of persistence or progression of CNS disease after 2 weeks of treatment or CNS reactivation<sup>1,9,11</sup>. HLH-2004 advises corticosteroids be added to intrathecal therapy<sup>11</sup>. As some patients develop asymptomatic CNS involvement<sup>8</sup>. As a minimum, CSF analysis is recommended at the time of onset, and during reactivation of systemic or neurological features<sup>11</sup>.

For patients with rheumatic disease and associated HLH, cyclosporin is recommended either as first line therapy or as a second line agent, if high dose corticosteroids do not achieve an adequate response<sup>8</sup>.

In MAHS, treatment depends on the clinical scenario. It is focused on treating the malignancy and possible infections in patients not yet on chemotherapy. If a child is on chemotherapy and the malignancy is controlled appropriate treatment might include an interruption of chemotherapy, along with treatment of infection, steroids and etoposide. In rapidly progressive MAHS initial therapy is aimed at controlling the acute cytokine storm with steroids and etoposide focusing on the treatment of the malignancy once the patient is more stable<sup>4</sup>.

Supportive therapy recommendations (HLH-2004) during the initial phase include, broad spectrum antibiotics when required, prophylactic oral co-trimoxazole, an oral antifungal, and possibly an oral antiviral agent. Immunoglobulins once every 4 weeks, and gastroprotective therapy (e.g. with Ranitidine) is also recommended<sup>11</sup>. A retrospective review of adverse outcomes of patients treated at the Hospital for Sick Children by Sung *et al* found that 50% of deaths were due to invasive fungal infection, emphasizing the need for a low threshold in starting antifungal treatment during prolonged neutropaenia, as well as prophylactic antifungal during chemotherapy<sup>23</sup>.

In patients with FHLH, genetic counseling and prenatal diagnosis in future pregnancies should be offered to the parents.

### **Prognosis**

The mortality in primary HLH is high. The disease is uniformly fatal in the absence of an HLA identical sibling bone marrow transplant, although chemotherapy alone may prolong survival<sup>1</sup>. On reviewing 122 children on the international registry Arico *et al* found a 5 year survival of 10% in children receiving chemotherapy alone and 66% for those who received an allogeneic bone marrow transplant<sup>24</sup>. Death may occur as a result of neutropaenic sepsis, multi-organ failure or CNS disease<sup>13</sup>. The median survival time without treatment is 2-6 months<sup>1,6,9</sup>.

In secondary phenomena the diagnosis and control of precipitating disease entities is crucial in arresting evolving clinical disease and preventing death. In a review of paediatric cases of IAHS reported between 1979-1996 (n= 219) by Janka *et al*<sup>4</sup>, the reported mortality rate was 38% in children older than three years old, 60% in children under three, but worse yet for those under

one year(68%). Amongst the infective precipitants, bacteria-related HLH had the best prognosis (82% survival), and EBV related, the worst (22% survival).

Using the treatment guidelines in HLH-94, 119 patients were followed up by Henter *et al* between 1994-1998. The 3 year overall survival was 55% for HLH and 51% for patients with FHLH. Patients who survived without a bone marrow transplant were all retrospectively presumed to have had non-genetic forms of the disease. No familial cases survived without transplant. A 64% 3 year overall survival was found in patients after haemopoietic cell transplant (varying from 71% for matched related donors to 50% for unmatched, unrelated donors)<sup>11</sup>.

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#### 4. Database Review

##### Methods

A retrospective review of data was performed using hospital folders of all children who met the diagnostic criteria for HLH (Table I) treated at Red Cross War Memorial Children's Hospital between March 1991 and September 2010. Cases were identified using the institutional Red Cross Oncology Registry.

All patients with Langerhans Cell Histiocytosis, malignant histiocytoses or those who did not meet the diagnostic criteria for HLH were excluded.

Prior to 1991 four patients who presented with histopathological features of a haemophagocytic syndrome were excluded, as there was no other record of serological identifiers for disease apart from cytopaenias.

##### Results

Fifteen patients met the criteria for the diagnosis of HLH. Eight of the patients were female, seven male. The median age at diagnosis was 27 months (10-79 months) for males and 75 months (9-225 months) for females. Henter *et al* reports a male: female ratio of 1:1<sup>1</sup>.

We did not classify any of our patients as having fHLH. There were multiple reasons we felt that secondary HLH was more likely:

- None of the patients included in the study had a documented family history of HLH, and no folders contained any history of consanguinity or unexplained family illnesses or deaths related to haematological problems (This does not exclude familial HLH, as it is an autosomal recessive trait<sup>1</sup>).
- The age of the patients: Of the three patients were under 1 year of age at diagnosis, the age at which primary HLH is likely to present<sup>1</sup>, two are alive disease free and one died of an unrelated cause. Two patients died of disease. These were both older than 12 months at the time of

diagnosis, an age they would have been unlikely to reach in the presence of severe immune deficiency.

- In most patients we had a reasonable degree of certainty that we had established a primary cause.
- In all patients with refractory disease, remission was eventually achieved by increasing the doses of steroids and/or adding a second or third agent and lengthening the eight week induction.

Ten patients are black, four of mixed ancestry and one patient is Bangladeshi. There are no Caucasians in our cohort. According to Henter *et al* HLH has been reported in many ethnic groups, from all continents<sup>1</sup>.

The median time to achieve a diagnosis once the children had arrived at our own institution was 13.7 days (1- 38 days) compared to seven days to four weeks reported in the literature<sup>15, 16</sup>. In certain cases the time to diagnosis was shortened due to referring units pre-emptively discussing the case with the Haematology/Oncology department. This undoubtedly shortened the interval to diagnosis, and the commencement of treatment.

#### Clinical features (see Table V)

Fever was documented in 13 (87%) patients. This is in keeping with previous studies with larger cohorts of patients (91-100%) as reviewed by Henter *et al*<sup>1</sup>. The actual duration of fever was only available for seven patients, and this included fever noted by parents and referral centres. The median duration of fever in these patients was 19.2.days (1-51 days). One patient was referred with a history of intermittent fever for 6 months.

Splenomegaly was documented in 10 (67%) patients compared to 97-100%<sup>1</sup>. Despite hepatomegaly not being a diagnostic criterion, it was present in 11(73%) patients compared to 94-97% in an earlier study<sup>1</sup>. Other common features were rash (7 [47%, compared to 6-65%<sup>1</sup>]) oedema (8) and lymphadenopathy (7, [47%, compared to 17-52%<sup>1</sup>]).

Five patients were found to have clinical CNS involvement which is lower than the 75% found in a series by Hallahan *et al*, although in most of these patients CNS features were not present initially but developed later in the clinical course<sup>16</sup>. Signs included non-specific pain, refusal to weight bear, abnormal gait, hydrocephalus, meningism, strabismus, hypotonia, weakness and neuroregression. One patient had no clinical CNS disease but had an abnormal CSF and meningeal enhancement on CT scan. In our setting meningeal enhancement is more commonly associated with meningitis, particularly tuberculous meningitis (TBM), but the CSF findings did not support a diagnosis of TBM.

### Precipitants

All of the patients included in the group were classified as having secondary HLH. Ten patients had clear precipitants. In three patients EBV was the precipitating cause (viral load log values of >4). One of these three patients also had SLE. Eleven patients had evidence of previous EBV (IgG positive or viral loads log values of 3.2 - 4). One patient had no evidence of EBV.

Other precipitants included CMV and RSV (1) in the same patient, pneumococcal endocarditis (1), Kawasaki disease (1), acute lymphoblastic leukaemia (on maintenance chemotherapy) (1), acute myeloid leukaemia (preceding diagnosis of malignancy) (1), anaplastic large cell lymphoma (preceding the diagnosis of malignancy) (1) and T- cell lymphoma (on maintenance chemotherapy) (1). No clear precipitant was found in the five remaining patients.

### Investigations

14 patients had documented serum ferritin levels at diagnosis. Thirteen had levels above 500mcg/L. The highest ferritin level was recorded was 10 000 mcg/L. This is useful as Allen *et al* found a ferritin level on admission of above 500mcg/L to be 100% sensitive for HLH<sup>15</sup>.

All patients had multiple full blood counts .The first values demonstrating an abnormality were recorded, and in many cases results continued to deteriorate before normalizing. HLH is a progressive disease and as such biochemical changes are dynamic. All 15 patients had haemoglobin levels of less than 11.5g/dl and 12 had a haemoglobin of 9.0g/dl or less. Henter *et*

*al* reported anaemia in 89-94% of patients<sup>1</sup>. Despite its sensitivity, low serum haemoglobin has a broad differential, so anaemia in isolation is not a particularly meaningful finding.

The median white cell count was  $17.2 \times 10^9/L$  (1 -  $63.1 \times 10^9/L$ ) and five patients had values under  $4 \times 10^9/L$ . The median platelet count was  $68 \times 10^9/L$  (13 -  $145 \times 10^9/L$ ).

Seven of 14 patients (50%) who had serum triglyceride levels tested had elevations in excess of 3mmol/L. None of the records reflected whether or not children were fasted prior to triglyceride levels being tested. Henter *et al* reported hypotriglyceridemia in 80-100% of patients<sup>1</sup>.

Six of the 13 patients who had recorded serum fibrinogen levels were below 1.5g/L. Sensitivity is reported as 53-64% in the literature<sup>3,8</sup>, but this test is useful as fibrinogen is usually increased in infection- often the primary differential diagnosis.

An elevated serum LDH of more than 1000 U/L is considered supportive evidence for HLH<sup>16</sup>. Only 3 of 15 patients in the cohort had elevated serum LDH levels.

Nine patients had lumbar punctures performed. Histiocytes were seen in the CSF of 3 patients, but no patients had an elevated CSF protein. CSF neuroleptin levels are not available at our institution.

All patients had bone marrow biopsies at diagnosis. All but one demonstrated haemophagocytosis with histiocytic infiltration. One patient was thought to have an immune thrombocytopenia based on a bone marrow done at five months of age, which showed an anaemia of chronic disorders and increased megakaryocytes with peripheral blood thrombocytopenia. She was admitted a second time at 14 months of age with sepsis. A second bone marrow then revealed HLH.

One patient had haemophagocytosis present on a lymph node, bone marrow and skin biopsy. She presented with superior vena caval syndrome and airway obstruction, which required intubation and ventilation. Subsequently a diagnosis of anaplastic large cell lymphoma was made. Three of the four patients who had liver biopsies demonstrated haemophagocytosis.

### Blood Product Support

Twelve patients required transfusions of packed cells. The median number of transfusions per patient was 9 (1-34 transfusions). Nine patients received platelet transfusions (1-18). Two patients received cryoprecipitate and three patients fresh frozen plasma.

### Treatment

Thirteen of the fifteen patients received steroid therapy. Two patients demised, one before any treatment could be commenced, and a second from an unknown cause at her referral hospital six months after discharge.

Of the thirteen, ten patients were started on dexamethasone, nine at 10 mg/m<sup>2</sup> and one patient (who was on maintenance therapy for T-cell lymphoma at the time) was started on 5mg/m<sup>2</sup>. Two patients were treated with prednisone. One patient was started on prednisone initially but was changed to dexamethasone (10mg/m<sup>2</sup>) nine days later.

Six patients were started on etoposide and two on cyclosporine (as per the HLH 2004 protocol) together with steroid therapy.

No patients came to bone marrow transplant.

### Outcomes

Ten patients are alive: six are disease free, one has refractory disease still on treatment, one with relapsed disease and two still on the induction phase of treatment. Four patients have died; one from refractory HLH, one from relapsed HLH, one from end stage HIV and one from an unknown cause. One patient was lost to follow up.

Of the three patients with relapsed disease, two have died of disease. The third patient relapsed after being treated with steroid alone and is currently on second line therapy with etoposide and cyclosporin.

## Discussion

The first patient with presumed HLH was diagnosed at Red Cross in 1973.

This patient, who has been excluded from the analysis, had a post mortem diagnosis made based on a histological finding of histiocytic infiltration with haemophagocytosis in multiple organs. He was four months old at the time of his death. His older sister who was living in England, suffered from a neurodegenerative disease characterized by acute febrile episodes with thrombocytopenia. Although extensively investigated the cause of her condition had not been found but was presumed to be due to a hereditary metabolic disorder. After her brother's death, the possibility of a haemophagocytic syndrome as an explanation for her symptoms was considered, and specimens of both siblings were re-examined. The sister died before a liver biopsy could be performed but retrospective comparison suggested that these patients may have suffered from a familial form of HLH. This diagnosis was made well before established diagnostic criteria were proposed or were in use.

The earliest patient included in this analysis was diagnosed in 1991. He retrospectively fulfilled 5 of the 8 criteria: documented fever, splenomegaly, cytopenias, haemophagocytic histiocytosis on bone marrow and liver biopsy and raised serum triglycerides (at that time the upper limit of normal for serum triglycerides was 1.6 according to the laboratory reference range). The child was treated with steroids, cyclosporin and etoposide and responded to therapy.

Formal diagnostic criteria were first proposed in 1994 and since that time fourteen patients have been diagnosed with HLH at Red Cross Hospital (ten of these in the last two years). This is a clear reflection of the increasing awareness amongst physicians of HLH as a distinct haematological entity.

An HIV positive patient who had defaulted highly active antiretroviral therapy, developed HLH and had positive cultures for multiple infections. Frequent infections are part of the clinical course of both HIV infection and HLH and in this patient, deciding which organism was the precipitant for HLH was challenging.

Similarly other illnesses such as Kawasaki disease can cause diagnostic difficulties because of similarities in presentation. One patient with Kawasaki disease did not respond to initial treatment with IVIG. She developed bicytopenia requiring multiple blood product transfusions and had a markedly raised LDH. Further investigations revealed hyperferritinaemia, raised triglycerides and hypoferritinaemia. A bone marrow showed features compatible with HLH<sup>25</sup>.

CNS involvement is reported in 73-75% of patients with HLH<sup>16,17</sup>. Our cohort did not display the high incidence reported in other series. Many patients were noted to be irritable, but this was usually attributed to fever and acute illness. This may imply that more subtle signs of CNS involvement may have been missed. Two patients were noted to have developmental regression, one prior to developing HLH and the other thereafter. This may reflect that the developmental history is typically not explored sufficiently when dealing with an acutely ill child or when referring patients with more pressing problems.

The two most consistent laboratory findings amongst these patients were anaemia and hyperferritinaemia. However, the differential diagnosis for anaemia is substantially broader, making a markedly elevated serum ferritin a far more reliable indicator of a possible histiocytic disorder.

It is our institutional policy to start dexamethasone monotherapy for non-EBV related HLH as most patients respond to steroids alone. Our institutional indications for etoposide and cyclosporine co-therapy are:

- Proven EBV- related HLH
- Refractory disease
- Relapsed disease
- Suspected primary HLH
- Critically ill patients with multi-organ failure, or life threatening complications

There are multiple reasons for this policy. One is the expense of cyclosporin, as well as the cost and difficulty involved in biweekly monitoring of patients in a setting such as ours where many

patients are referred from distant areas and where space and time constraints within the hospital pose very real challenges.

Although the development of diagnostic criteria has greatly assisted paediatricians in diagnosis of HLH, there are limitations in developing countries.

Firstly, there is a bias towards laboratory criteria. Resource constraints limit the usefulness of diagnostic criteria that are very laboratory dependant. Limited facilities also affect the time taken to obtain results, make a diagnosis and commence treatment. For example, soluble IL-2 receptor levels and NK-activity tests are not available in South Africa. Patients therefore need to fulfill five of the remaining six criteria to make a diagnosis of HLH.

In addition, the disease process is dynamic. Diagnostic criteria are always limited to capturing a patient at a specific moment in time. Although a patient may not meet the criteria at first presentation, he or she may do so at a later stage as the disease progresses. Serial examinations and investigations may be needed to make the diagnosis. This may not be practical in a setting where many patients are required to travel vast distances to referral hospitals.

Diagnostic guidelines which rely more heavily on clinical criteria would assist doctors in resource constrained environments. This is the case for a newly proposed modification of the guidelines (see Table II) which, although using many of the same criteria, is also less stringent, allowing for a diagnosis of 'suspected' HLH.

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## 6. Appendix

### Addendum A: Diagnostic Criteria

**Table I. Revised Diagnostic Guidelines for HLH**

The diagnosis HLH can be established if one of either 1 or 2 below is fulfilled

1. A molecular diagnosis consistent with HLH

2. Five of the eight criteria below are met:

A) Initial diagnostic criteria (to be evaluated in all patients with HLH)

- Fever
- Splenomegaly
- Cytopenia affecting 2 or more lineages
  - Hb < 9g/dL ( in neonates < 10g/dL)
  - Platelets <  $100 \times 10^9/L$
  - Neutrophils <  $1.0 \times 10^9/L$
- Hypertriglyceridaemia – fasting triglycerides  $\geq 3$  mmol/L  
and/or  
hypofibrinogenemia – fibrinogen  $\leq 1.5$  g/L
- Haemophagocytosis in bone marrow, spleen or lymph nodes,  
No evidence of malignancy

B) New diagnostic criteria

- Low or absent NK-cell activity (according to local laboratory reference)
- Ferritin > 500 mcg/L
- Soluble IL-2 receptor  $\geq 2400$  U/mL

#### Comments

- If haemophagocytic activity is not proven at the time of presentation, further search for haemophagocytic activity is encouraged. If the bone marrow specimen is not conclusive, material may be obtained from other organs. Serial marrow aspirates over time may also be helpful.
- The following findings may provide strong supportive evidence for the diagnosis: a) spinal fluid pleocytosis (mononuclear cells) and/ or elevated spinal fluid protein, b) histological picture in the liver resembling chronic persistent hepatitis (biopsy).
- Other abnormal clinical and laboratory findings consistent with the diagnosis are: cerebromeningeal symptoms, lymph node enlargement, jaundice, oedema, skin rash. Hepatic enzyme abnormalities, hypoproteinemia, hyponatremia, VLDL increased, HDL decreased.

Henter *et al*<sup>1</sup>

**Table II. Proposed Diagnostic Criteria, 2009**

1. Molecular diagnosis of haemophagocytic lymphohistiocytosis (HLH) *Or*

X- linked lymphoproliferative syndromes (XLP)

2. Or at least 3 of 4:

- Fever
- Splenomegaly
- Cytopenias (minimum 2 cell lines reduced)
- Hepatitis

3. And at least 1 of 4:

- Haemophagocytosis
- Increased ferritin
- Increased soluble IL-2 receptor
- Absent or very decreased NKC function

4. Other results supportive of HLH diagnosis

- Hypertriglyceridaemia
- Hypofibrinogenaemia
- Hyponatraemia

Filipovich<sup>26</sup>

## Addendum B: Patient Cohort

**Table III. Patient Data**

<b>Patient Number</b>	<b>Gender</b>	<b>Ethnicity</b>	<b>Age at diagnosis (in months)</b>	<b>Precipitant</b>	<b>Outcome</b>
1	Male	Mixed ancestry	27	Pneumococcal endocarditis	ADF
2	Male	Black	15	AML	DD
3	Male	Mixed ancestry	79	ALL	DD
4	Male	Black	14	CMV, RSV	AWD
5	Male	Black	10	NCC	AWD
6	Male	Black	11	EBV	DU
7	Male	Black	36	EBV	ADF
8	Female	Mixed ancestry	20	NCC	ADF
9	Female	Black	68	NCC	DU
10	Female	Black	33	NCC	Lost
11	Female	Black	147	T cell lymphoma	ADF
12	Female	Bangladeshi	26	NCC	ADF
13	Female	Mixed ancestry	73	Kawasaki Disease	ADF
14	Female	Black	9	Anaplastic large cell lymphoma	AWD
15	Female	Black	225	SLE,EBV	AWD

**NCC** No clear cause, **ADF** Alive disease free, **DD** Died of disease, **AWD** Alive with disease,

**DU** death unrelated **Lost** Lost to follow up

**Table IV. Patient Biochemical / Diagnostic Markers**

Patient Number	Ferritin (mcg/L)	Triglyceride (mmol/L)	Fibrinogen (g/L)	LDH (U/L)	ESR (mm/hr)	Hb (g/dL)	WBC (x10 <sup>9</sup> /L)	Plts (x10 <sup>9</sup> /L)	Histiocyte Infiltration / haemophagocytosis	EBV VL (log value)
<b>Normal values<sup>22</sup></b>	M: 7-300 F: 7-120 *	<b>M: 0.31-1.4 F: 0.38- 1.4</b>	<b>M: 1.8-3.4 F: 1.9-4.2</b>	<b>150-360</b>	<b>M: 0-10 F: 0-15</b>	<b>M: 10.5-18 F: 10.5-16 *</b>	<b>6-10.5*</b>	<b>140-340</b>		
<b>Diagnostic criteria</b>	>500	>3.0	<1.6			<9.0	<5	<100		
1	<b>6502</b>	<b>4.6</b>	<b>1</b>	608	5	<b>4.5</b>	<b>18.8</b>	<b>31</b>	BM	3.2
2	<b>4193</b>	-	<b>0.6</b>	533	<b>72</b>	<b>2.9</b>	<b>2.2</b>	<b>13</b>	BM , CSF, LB	-
3	<b>1953</b>	<b>4.2</b>	<b>1.1</b>	348	1	9	<b>2.6</b>	<b>39</b>	BM	<b>4.8</b>
4	<b>885</b>	2.3	3.8	272	4	<b>8.3</b>	<b>22.3</b>	<b>53</b>	BM	-
5	<b>9424</b>	2.8	3	<b>3497</b>	<b>62</b>	9	9.9	141	BM, CSF	3.3
6	<b>811</b>	1.5	<b>0.1</b>	590		<b>3.2</b>	<b>35.1</b>	<b>16</b>	BM	<b>6.5</b>
7	<b>1533</b>	2.3	6.4	467	<b>150</b>	<b>3.6</b>	<b>26.7</b>	<b>94</b>	BM	3.7
8	<b>4047</b>	<b>6.1</b>	<b>0.3</b>	<b>1134</b>	<b>125</b>	<b>7.3</b>	<b>31.2</b>	109	BM	3.7
9	291	1.4	2.3	893	<b>40</b>	<b>8.6</b>	<b>3.4</b>	115	BM	-
10	-	1.9	-	120	-	<b>4.3</b>	4.9	<b>22</b>	BM, LB	-
11	<b>4174</b>	<b>5.5</b>	2.8	270	<b>102</b>	11.3	<b>1</b>	<b>31</b>	BM	<b>4</b>
12	<b>4338</b>	<b>8.4</b>	2.1	935	<b>35</b>	10.4	<b>15.7</b>	101	BM	3.3
13	<b>10000</b>	<b>3.5</b>	<b>0.8</b>	<b>4873</b>	<b>19</b>	<b>8</b>	<b>27.2</b>	<b>91</b>	BM	<b>4</b>
14	<b>4635</b>	<b>5.4</b>	-	224	-	10	<b>63.1</b>	<b>23</b>	BM, LN	-
15	<b>567</b>	2.7	6.2	287	<b>75</b>	<b>8.1</b>	<b>3.6</b>	145	BM, CSF	<b>4.5</b>

**M** male, **F** female, **LDH** Lactate dehydrogenase, **ESR** Erythrocyte sedimentation rate, **BM** Bone marrow, **CSF** Cerebrospinal fluid, **LB** Liver biopsy, **LN** Lymph node biopsy, **VL** Viral load, **WBC** White Blood Count, **Plts** Platelets.

*Numbers highlighted indicate abnormal values relative to the diagnostic criteria. WBC seen is the total white blood count and not the total neutrophil count. EBV VL > 4 log considered significantly elevated.*

*\*Reference ranges variable dependant on age and gender. The range stated is for children from 9-225 months of age both male and female.*

**Table V. Clinical features**

No.	Fever (in days)	Hepar	Spleen	Skin	Oedema	LN	CNS	Cardiac	Resp	Haem	Other
1	unknown	+	+		+			+			+
2	unknown	+	+	+		+	+		+	+	
3	No fever				+		+		+		
4	No fever			+					+		+
5	10	+	+	+		+			+	+	+
6	19	+	+	+	+	+		+	+		+
7	1	+	+	+		+			+		+
8	51	+	+		+	+					
9	16	+	+		+						+
10	unknown	+	+		+					+	
11	unknown						+				
12	21	+	+	+		+					+
13	17			+	+		+	+			
14	unknown	+	+	+	+	+	+		+		
15	unknown	+					+		+		

**Hepar** Hepatomegaly, **Spleen** Splenomegaly, **Skin** involvement included rash, subcutaneous nodules or any documented skin lesions, **LN** Lymph nodes, **CNS** involvement included meningism, hydrocephalus, headache, hypertonia, twitching, abnormal gait, lethargy, hypotonia, dizziness, neuroregression, unexplained pain, strabismus, decreased level of consciousness, **Cardiac** signs included murmur, cardiac failure, **Resp** respiratory symptoms including cough, airway obstruction and unspecified respiratory illness, **Haem** haematological involvement included bleeding, petechiae, **Other features** include joint swelling, diarrhoea, conjunctivitis, wasting, abdominal distention, jaundice

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3. Virchow R. *Cellular Pathology*. Philadelphia: JB Lippincott, 1863.

#### **Software**

4. Epi Info [computer program]. Version 6. Atlanta, GA: Centers for Disease Control and Prevention; 1994.

#### **Online Journals**

5. Friedman SA. Preeclampsia: a review of the role of prostaglandins. *Obstet Gynecol* [serial online]. January 1988;71:22–37. Available from: BRS Information Technologies, McLean, VA. Accessed December 15, 1990.

#### **Database**

6. CANCERNET-PDQ [database online]. Bethesda, MD: National Cancer Institute; 1996. Updated March 29, 1996.

#### **World Wide Web**

7. Gostin LO. Drug use and HIV/AIDS [JAMA HIV/AIDS Web site]. June 1, 1996. Available at: <http://www.ama-assn.org/special/hiv/ethics>. Accessed June 26, 1997.

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## 8. Publishable Journal Article

### **Serum Ferritin is a Cost Effective Laboratory Marker for Haemophagocytic Lymphohistiocytosis in the Developing World**

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**Abstract**

Haemophagocytic Lymphohistiocytosis (HLH) is a rare disease in children<sup>1</sup>, and presents many diagnostic difficulties. Without prompt intervention, the disease typically runs a rapidly fatal course. Diagnostic criteria were proposed by the Histiocyte Society in 1991 and have since been modified. Included in these criteria is a ferritin level  $> 500\text{mcg/L}^2$ . Although not diagnostic, a high ferritin level is highly suggestive of HLH. Serum ferritin assays are more accessible and cost effective compared to other biochemical markers, particularly in resource limited settings.

Fifteen patients with HLH were treated at Red Cross War Memorial Children's Hospital between 1991 and 2010. Hyperferritinaemia was a consistently reliable finding (93%) compared to either serum fibrinogen or triglycerides which were elevated in only half of the patients. It is our contention that a complete blood count and serum ferritin (in addition to clinical criteria and tissue examination of marrow and / or CSF) is probably the single most cost effective and clinically helpful means to make the diagnosis of HLH where laboratory access is limited.

Key words: haemophagocytic lymphohistiocytosis, ferritin

## Introduction

Haemophagocytic Lymphohistiocytosis (HLH) is a rare disease in children with an estimated incidence of 0.12 cases per 100 000 children<sup>1</sup>. As no published series is available, the incidence in Africa is unknown. Without treatment the disease runs an often fatal course, making prompt diagnosis a priority. The diagnosis may be difficult since HLH is a diagnosis of exclusion with no pathognomonic features, and a clinical picture which may be vague and varied. For this reason it is usually not considered early as a differential diagnosis to exclude and so treatment delays are inevitable.

In order to aid diagnosis, a set of diagnostic criteria including clinical and biochemical markers was proposed by the Histiocyte Society in 1991<sup>2</sup>. These were modified in 2004 (Table I), and new recommendations have been made since but have not yet been codified (Table II). Included in these criteria is a ferritin level > 500mcg/l<sup>2</sup>. In a study by Allen *et al* a ferritin level >10000mcg/l was found to be 90% sensitive and 96% specific for HLH (98% if fever added as a criterion)<sup>3</sup>. In a series of patients including both viral- and malignancy associated HLH, Esumi *et al* found ferritin levels to be elevated at diagnosis, with further increases at the onset of DIC. Patients with sustained high ferritin levels after three months died, while those with decreased levels survived, suggesting that ferritin level may be a useful marker to monitor disease activity and not just as an indicator of the presence of disease<sup>4</sup>. Serum ferritin levels are more readily available in most laboratories compared to functional NK assays or IL-2 alpha receptor serum assays, which is of particular importance in Africa where laboratory services are often limited or unavailable.

Ferritin production is up-regulated by pro-inflammatory cytokines from the NK pathway<sup>3</sup>, the activated yet ineffective pathway in HLH. The differential diagnosis for conditions associated with hyperferritinaemia include other infections, auto-immune disease, like juvenile systemic arthritis<sup>5</sup>, and several very rare syndromes which include lysinuric protein intolerance (LPI), congenital haemochromatosis and hereditary hyperferritinaemia-cataract syndrome<sup>6</sup>. A high ferritin level alone is therefore very suggestive but not diagnostic of HLH.

## Materials and Methods

Between March 1991 and September 2010 fifteen patients were diagnosed with HLH at Red Cross War Memorial Children's Hospital. This diagnosis was made based on the HLH diagnostic criteria (Table I). These criteria were modified for our local use to include an abnormal CSF with other tissues like the bone marrow and lymph nodes. This was done because CSF changes may be present even without any clinical features<sup>5</sup> or conversely CSF may appear normal, even in patients with symptomatic CNS involvement<sup>7</sup>. For this reason, Janka *et al* recommend a lumbar puncture be done in all patients<sup>5</sup> as CNS involvement is associated with a worse outcome<sup>7</sup>. Also, NKC activity testing and IL2 levels are not available at our institution. Cases were identified using the institutional oncology registry, and a retrospective review of data was performed using hospital folders.

Prior to 1991 four patients had histopathological features compatible with a haemophagocytic syndrome. These patients presented prior to there being specific diagnostic recommendations, as such many of the investigations required to meet the criteria were not performed, resulting in insufficient evidence for the diagnosis to be made. These patients, as well as those with Langerhans cell histiocytosis, malignant histiocytosis or those who did not meet the diagnostic criteria for HLH were excluded from this series.

## Results

Eight of the 15 patients were female and seven male. The median age at diagnosis of 27 months (10-79 months) for males and 75 months (9-225 months) for females. Three patients were under 1 year of age at diagnosis, the age when primary HLH is more likely to present<sup>6</sup>.

Ten patients are black, four of mixed ancestry and one patient is Bangladeshi. There are no Caucasians in our cohort. None of the patients included in the study had a documented family history of HLH, although none of the folders contained any specific history of consanguinity or unexplained family illnesses or deaths related to haematological problems. All of the patients included in the group were classified as having secondary HLH. Three patients had EBV infection (viral load log values of >4) as a precipitant.

Precipitants were varied and included CMV and RSV (1) and pneumococcal endocarditis (1), Kawasaki disease (1), SLE (1). Four patients had malignancy-associated HLH: ALL (1), AML (1), T-cell lymphoma (1), and anaplastic large cell lymphoma (1).

The median time to achieve a diagnosis once the children had arrived at our own institution was 13.7 days (1- 38 days). Discussion prior to referral undoubtedly shortened the interval to diagnosis in certain cases.

Fever was documented in 13 (87%) patients. Splenomegaly was documented in 10(67%) patients. Despite hepatomegaly not being a diagnostic criterion, it was present in 11(73%) patients. Other common features were rash (7,[47%]), oedema (8,[54%]) and lymphadenopathy (7,[47%]). By comparison, Henter *et al* found the following clinical features of patients in a literature review, a population based study and the FHL Registry , respectively<sup>1</sup> : fever (91%, 100%, 93%), splenomegaly (98%, 100%, 97%), hepatomegaly (94%, 97%, no data available) rash (6%, 65%, 24%), lymphadenopathy (17%, 52%, 31%). Five patients were found to have clinical CNS involvement which is lower than the 75% found in a series by Hallahan *et al*, although in most of these patients CNS features were not present initially but developed later in the clinical course<sup>7</sup>.

Of 14 patients who had documented serum ferritin levels, 13 met the diagnostic criterion of levels above 500mcg/L.

All patients had multiple complete blood counts. The first values demonstrating an abnormality were recorded, and in many cases results continued to deteriorate before normalising. Twelve of 15 had serum haemoglobin levels of 9.0g/dL or less. Five patients had total white blood count values under  $4 \times 10^9/L$  and 10 had platelet counts of less than  $100 \times 10^9/L$  (13 –  $145 \times 10^9/L$ ). Seven of 14 patients who had serum triglyceride levels tested had elevations in excess of 3mM/L. Six of 13 patients had fibrinogen levels below 1.5g/L.

All patients had bone marrow biopsies at diagnosis. All bar one demonstrated haemophagocytosis with histiocytic infiltration on initial or follow up biopsy. One patient had haemophagocytosis present on a lymph node biopsy. Three of the four patients who had liver biopsies demonstrated haemophagocytosis (Table III).

Thirteen patients were commenced on steroids. Ten were started on dexamethasone, two on prednisone. One of the patients started on prednisone initially was changed to dexamethasone nine days later.

Six patients were started on etoposide and two on cyclosporin (as per the HLH 2004 protocol).

Whether or not patients received etoposide or not did not seem to have any bearing on outcome, keeping in mind that it is a small cohort of patients and that our criteria for etoposide were fairly strict (primary steroid non-responders, proven EBV associated HLH, refractory or reactivated HLH).

No patients required bone marrow transplant.

Four patients have died; one from refractory HLH, one from reactivated HLH, one from end stage HIV infection and one from an unknown cause. Ten patients are still alive; six are disease free and four are alive with disease: one with refractory disease on treatment, one with reactivated disease on second line treatment and two still on the induction phase of treatment. One patient has been lost to follow up.

## **Discussion**

The diagnosis of HLH in resource constrained environments is made more difficult by the reliance on laboratory criteria which are often unavailable or limited. New proposed modifications to the diagnostic criteria (Table II) will help to make this less so, as they are somewhat less laboratory focused compared to the current criteria (Table I). However, the onus is still on the clinician to actively make the diagnosis, and to pursue treatment early on.

The costs related to specific investigations are often not considered by the user. Cost considerations often move local, national or institutional groups to relook at diagnostic and treatment criteria, in order to balance what is best for the patient against what is affordable for a service provider. South Africa, although arguably more fortunate than most African countries, is not exempt to these pressures and we have had to make institutional alterations to the treatment guidelines for patients with HLH because of the costs of tests (cyclosporin levels) and drugs (cyclosporin, in particular). Furthermore these “hard” costs do not take include hidden expenses of difficulty with access, transport and accommodation for poor patients, especially those required to attend outpatient clinics several times a week. Table IV has a list of the cost of the laboratory tests typically involved in the diagnosis and followed up in HLH.

Because the disease is a dynamic process, investigations may need to be repeated before criteria are met and a diagnosis is made. Laboratory facilities themselves may be limited in

some hospitals and some tests not available at all (for example, soluble IL-2 receptor levels and NKC activity).

This then raises the question as to what the most cost effective and clinically instructive strategy is. In our series, hyperferritinaemia was a consistently reliable finding (93%) compared to either serum fibrinogen or triglycerides which were elevated in only half of the patients. This finding is supported by others studies in which a ferritin level >500 mcg/l was found in 100% of patients with HLH<sup>3</sup>.

Taking clinical comparators into account, fever was found in 87% of patients, hepatomegaly (73%) and anaemia (67%) were common findings but the differential diagnosis for each of these, or even in combination, remains much wider than for those with significant hyperferritinaemia, particularly in our region with high rates of endemic tuberculosis and HIV infection.

It is our contention that a complete blood count and serum ferritin (in addition to clinical criteria and tissue examination of marrow with or without a CSF ) is probably the single most cost effective and clinically helpful means to make the diagnosis and that serum fibrinogen and /or triglycerides are useful adjuncts, when there is still sufficient doubt about the diagnosis. A full set of investigations (complete blood count, fibrinogen, ALT, LDH, triglycerides, ferritin, bone marrow aspiration) will cost roughly \$130 US per patient. Based on our small group of patients it seems reasonable to limit investigations to a complete blood count, serum ferritin and bone marrow biopsy examination (at a cost of approximately \$95 US), and initially forego the others, considering that the ferritin is consistently more reliable as an indicator of disease, compared to serum triglycerides and fibrinogen; a cost saving of approximately \$35 US per patient.

Rationalizing costs should be a priority in any clinical care setting, and often measures taken to limit costs that arise out of necessity, subsequently have real implications for how patients are managed in environments even where resources are not limited.

There are a number of weaknesses of our review. The small size of the cohort limits it's power and thus the interpretation of the findings. Although we believe some of the excluded patients did have HLH, they did not meet our inclusion criteria. Our inability to do certain tests due to cost and availability may have negatively affected the size of the cohort. Resource limitation

also had an impact on what upfront treatment could be offered. Some patients responded adequately to steroids alone, which may imply an immunological phenotype which is different to those who do not. We are unable to pre-empt which patients as these more sophisticated tests are not available at our institution. Survival analysis was not possible since some of the patients were still on treatment at the time of the analysis

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## Table I. Revised Diagnostic Guidelines for HLH

The diagnosis HLH can be established if one of either 1 or 2 below is fulfilled

1. A molecular diagnosis consistent with HLH

2. Five of the eight criteria below are met:

A) Initial diagnostic criteria (to be evaluated in all patients with HLH)

- Fever
- Splenomegaly
- Cytopaenia affecting 2 or more lineages
  - Hb < 9g/dL ( in neonates < 10g/dL)
  - Platelets <  $100 \times 10^9/L$
  - Neutrophils <  $1.0 \times 10^9/L$
- Hypertriglyceridaemia – fasting triglycerides  $\geq 3$  mmol/L  
*and/or*  
hypofibrinogenemia – fibrinogen  $\leq 1.5$  g/L
- Haemophagocytosis in bone marrow, spleen or lymph nodes,  
No evidence of malignancy

B) New diagnostic criteria

- Low or absent NK-cell activity (according to local laboratory reference)
- Ferritin > 500 mcg/L
- Soluble IL-2 receptor  $\geq 2400$  U/mL

### Comments

- If haemophagocytic activity is not proven at the time of presentation, further search for haemophagocytic activity is encouraged. If the bone marrow specimen is not conclusive, material may be obtained from other organs. Serial marrow aspirates over time may also be helpful.
- The following findings may provide strong supportive evidence for the diagnosis: a) spinal fluid pleocytosis (mononuclear cells) and/ or elevated spinal fluid protein, b) histological picture in the liver resembling chronic persistent hepatitis (biopsy).
- Other abnormal clinical and laboratory findings consistent with the diagnosis are: cerebromeningeal symptoms, lymph node enlargement, jaundice, oedema, skin rash. Hepatic enzyme abnormalities, hypoproteinemia, hyponatremia, VLDL increased, HDL decreased.

Henter *et al*<sup>2</sup>

**Table II. Proposed Modifications to Diagnostic Criteria, 2009**

1. Molecular diagnosis of haemophagocyte lymphohistiocytosis (HLH)

*Or X- linked lymphoproliferative syndromes (XLP)*

2. Or at least 3 of 4:

- Fever
- Splenomegally
- Cytopenias (minimum 2 cell lines reduced)
- Hepatitis

3. And at least 1 of 4:

- Haemophagocytosis
- Increased Ferritin
- Increased soluble IL-2 receptor
- Absent or very decreased NKC function

4. Other results supportive of HLH diagnosis

- Hypertriglyceridaemia
- Hyperfibrinogemia
- Hyponatraemia

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**Table III. Patient results**

No	Age months	Outcome	Ferritin mcg/L	TG mM/L	Fib g/L	Hb g/dL	WBC x10 <sup>9</sup> /L	Plts x10 <sup>9</sup> /L	Histiocyte infiltration & Haemophagocytosis	EBV VL (log)	Treatment
			>500	>3.0	<1.6	<9.0		<100			
<b>1</b>	<b>27</b>	<b>ADF</b>	<b>6502</b>	<b>4.6</b>	<b>1</b>	<b>4.5</b>	<b>18.8</b>	<b>31</b>	<i>bone marrow</i>	<b>3.2</b>	<b>D</b>
<b>2</b>	<b>15</b>	<b>DD</b>	<b>4193</b>	-	<b>0.6</b>	<b>2.9</b>	<b>2.2</b>	<b>13</b>	<i>bone marrow, CSF, liver biopsy</i>	-	<b>P,E</b>
<b>3</b>	<b>79</b>	<b>DD</b>	<b>1953</b>	<b>4.2</b>	<b>1.1</b>	<b>9</b>	<b>2.6</b>	<b>39</b>	<i>bone marrow</i>	<b>4.8</b>	<b>D, E</b>
<b>4</b>	<b>14</b>	<b>AWD</b>	<b>885</b>	<b>2.3</b>	<b>3.8</b>	<b>8.3</b>	<b>22.3</b>	<b>53</b>	<i>bone marrow</i>	-	<b>D, E</b>
<b>5</b>	<b>10</b>	<b>AWD</b>	<b>9424</b>	<b>2.8</b>	<b>3</b>	<b>9</b>	<b>9.9</b>	<b>141</b>	<i>bone marrow, CSF</i>	<b>3.3</b>	<b>D</b>
<b>6</b>	<b>11</b>	<b>DU</b>	<b>811</b>	<b>1.5</b>	<b>0.1</b>	<b>3.2</b>	<b>35.1</b>	<b>16</b>	<i>bone marrow</i>	<b>6.5</b>	-
<b>7</b>	<b>36</b>	<b>ADF</b>	<b>1533</b>	<b>2.3</b>	<b>6.4</b>	<b>3.6</b>	<b>26.7</b>	<b>94</b>	<i>bone marrow</i>	<b>3.7</b>	<b>D</b>
<b>8</b>	<b>20</b>	<b>ADF</b>	<b>4047</b>	<b>6.1</b>	<b>0.3</b>	<b>7.3</b>	<b>31.2</b>	<b>109</b>	<i>bone marrow</i>	<b>3.7</b>	<b>D, P</b>
<b>9</b>	<b>68</b>	<b>DU</b>	<b>291</b>	<b>1.4</b>	<b>2.3</b>	<b>8.6</b>	<b>3.4</b>	<b>115</b>	<i>bone marrow</i>	-	-
<b>10</b>	<b>33</b>	<b>Lost</b>	-	<b>1.9</b>	-	<b>4.3</b>	<b>4.9</b>	<b>22</b>	<i>bone marrow, liver biopsy</i>	-	<b>P,E</b>
<b>11</b>	<b>147</b>	<b>ADF</b>	<b>4174</b>	<b>5.5</b>	<b>2.8</b>	<b>11.3</b>	<b>1</b>	<b>31</b>	<i>bone marrow</i>	<b>4</b>	<b>D</b>
<b>12</b>	<b>26</b>	<b>ADF</b>	<b>4338</b>	<b>8.4</b>	<b>2.1</b>	<b>10.4</b>	<b>15.7</b>	<b>101</b>	<i>bone marrow</i>	<b>3.3</b>	<b>D</b>
<b>13</b>	<b>73</b>	<b>ADF</b>	<b>10000</b>	<b>3.5</b>	<b>0.8</b>	<b>8</b>	<b>27.2</b>	<b>91</b>	<i>bone marrow</i>	<b>4</b>	<b>D</b>
<b>14</b>	<b>9</b>	<b>AWD</b>	<b>4635</b>	<b>5.4</b>	-	<b>10</b>	<b>63.1</b>	<b>23</b>	<i>bone marrow, lymph node biopsy</i>	-	<b>D,C,E</b>
<b>15</b>	<b>225</b>	<b>AWD</b>	<b>567</b>	<b>2.7</b>	<b>6.2</b>	<b>8.1</b>	<b>3.6</b>	<b>145</b>	<i>bone marrow, CSF</i>	<b>4.5</b>	<b>D,C,E</b>

**ADF** alive disease free, **DD** died of disease, **AWD** alive with disease, **DU** death unrelated, **CSF** cerebrospinal fluid, **VL** viral load, **TG** triglycerides, **Fib** Fibrinogen. **D** Dexamethasone, **P** Prednisone, **E** Etoposide, **C** Cyclosporin

Numbers highlighted indicate abnormal values relative to the diagnostic criteria. WBC seen is the total white blood count and not the total neutrophil count. EBV VL > 4 log considered significantly elevated.

**Table IV. Costs of Laboratory Tests**  
**National Health Laboratory Services, South Africa**

<b>Test</b>	<b>Cost in SA Rands</b>	<b>Cost in US Dollars</b>
<b>Complete Blood Count</b>	107.00	15.98
<b>Fibrinogen</b>	36.70	5.45
<b>ALT</b>	55.10	8.20
<b>LDH</b>	55.10	8.20
<b>Triglyceride</b>	80.80	12.02
<b>Ferritin</b>	126.70	18.85
<b>Bone marrow examination</b>	395.82	58.77
<b>TOTAL (All tests)</b>	<b>R857.22</b>	<b>\$127.47</b>
<b>TOTAL (CBC+ Ferritin+BM)</b>	<b>R629.52</b>	<b>\$93.60</b>
<b>TOTAL saving</b>	<b>R227.70</b>	<b>\$33.87</b>

US Dollar: SA Rand exchange at time of calculation: 1: 6.7204

9. Proof of submission

Submissions Being Processed for Author Juli Renate Switala, MBBCh

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Action Links		Serum Ferritin is a Cost Effective Laboratory Marker for Haemophagocytic Lymphohistiocytosis in the Developing World	Jun 20 2011 6:14PM	Jun 20 2011 6:14PM	Submitted to Journal	Submitted to Journal

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