**Retrospective single-centre analysis of diagnostic approach to adult-onset
haemophagocytic lymphohistiocytosis**

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Abstract

Haemophagocytic lymphohistiocytosis (HLH) is rare disorder characterised by immune activation leading to cytokine storm and end-organ failure. It is associated with poor prognosis. HLH is predominantly a disease of early childhood, where it often results from genetic defects that impair cytotoxic function of natural killer cells and T cells. In adults, clinical manifestations are similar to those observed in children, although the aetiology is often unclear. We hypothesised that the poor prognosis is in part due to lack of awareness of the disorder, which results in incomplete investigation and failure to implement timely treatment. We evaluated diagnosis of HLH according to standard criteria in a single centre. In this retrospective case series, we present the clinical characteristics, treatment, and outcome of a cohort of adult-onset HLH identified in a tertiary hospital in Australia. In our cohort, hyperferritinaemia $\geq 10,000$ $\mu\text{g/L}$ was highly sensitive in detecting patients with adult-onset HLH, however the majority of patients who had hyperferritinaemia $\geq 10,000$ $\mu\text{g/L}$ did not have adult-onset HLH. We highlight that incomplete assessment of HLH criteria is frequently seen. Greater awareness of this rare disease and its diagnostic criteria may improve patient outcomes.

Introduction

Primary HLH arises as a result of mutations or deletions affecting *PRF1* (familial haemophagocytic lymphohistiocytosis (FHL)2), *UNC13D* (FHL3), *STX11* (FHL4), *STXBP2* (FHL5), *RAB27A* (Griscelli syndrome, type 2), *SH2D1A* (X-linked lymphoproliferative syndrome (XLP) type 1), *XIAP* (XLP2), or *LYST* (Chediak Higashi syndrome, CHS) .^{(1), (2, 3)}

Some cases of apparent primary HLH remain unexplained by genetic defects (eg FHL1).⁽³⁾ The mutations that have been identified so far result in impaired lymphocyte cytotoxicity.⁽⁴⁾ Secondary HLH refers to cases arising as a result of acquired immune dysregulation, malignancy, autoimmune disease (also known as macrophage activation syndrome, MAS), transplantation, and severe infection.⁽⁵⁾ The distinction between primary and secondary HLH is not always straightforward, as not all paediatric cases are accounted for by known mutations, and HLH may be the presenting illness in secondary cases.

HLH carries a poor prognosis. With use of the best validated treatment regimen (HLH-2004 protocol for children), which comprises combination therapy with cyclosporin, etoposide, and dexamethasone, the probability of survival at 5-years is estimated at 61% .⁽⁶⁾ In adults, treatment often consists of a modified version of this regimen, but outcome data are sparse. Early diagnosis is often difficult in absence of a sensitive and specific laboratory investigation tool that is cheap and readily available. Incorrect or late diagnosis may contribute to poor outcomes. Furthermore, other conditions including overwhelming sepsis and haematological malignancy can mimic the presentation of HLH, which contributes to the diagnostic dilemma.

Diagnostic criteria for HLH have been validated, and consist of fever, splenomegaly, bicytopenia, hyperferritinaemia, hypertriglyceridaemia/ hypofibrinogenaemia, tissue haemaphagocytosis, elevated soluble CD25, and reduced natural killer (NK) cell function.⁽¹⁾ Based on the HLH-2004 trial, five of eight criteria are required for a definitive diagnosis. Alternatively, the diagnosis can be achieved by identifying a mutation known to cause HLH.¹

Although a serum ferritin of $\geq 500 \mu\text{g/L}$ is widely used as one of the eight diagnostic criteria for HLH,¹ this was based on data from paediatric populations. Further studies found that ferritin $\geq 10,000 \mu\text{g/L}$ was 90% sensitive and 96% specific for the diagnosis of paediatric HLH.⁷ In contrast, recent case series demonstrated that use of an even more stringent definition of hyperferritinaemia ($\geq 50,000 \mu\text{g/L}$) is not sufficiently specific for a diagnosis of HLH in adults.⁸ This observation illustrates how extrapolating criteria from paediatric to adult population is problematic, possibly reflecting differences in causes of HLH between children and adults. At present, there is no consensus on adult-specific HLH diagnostic criteria.

We present the clinical characteristics, treatment, and outcome of a cohort of patients with adult-onset HLH from a tertiary hospital in Australia with the aim of improving recognition and treatment of this severe illness.

Methods

Patients and selection criteria

This study was approved by ACT Health Human Research Ethics and Governance Office (ETHLR.17.057). Case ascertainment was based on identification of patients with a discharge diagnosis of HLH or MAS from the Canberra hospital based on ICD-10 code (D76.1, haemophagocytic lymphohistiocytosis; D76.2, infection-driven haemophagocytic syndrome and D76.3, other histiocytosis syndrome, which was included in case HLH patients were mis-coded) between January 2007 and January 2017.).

Ferritin, serum soluble CD25 (sCD25), natural killer (NK) cell function and bone marrow results were obtained from the pathology database (ACT Pathology) that serve the same tertiary referral hospital (in addition to ACT region) during the same study period (**Fig. 1**). From this dataset, patients were included for further evaluation by HLH diagnostic criteria (2004 Histiocyte Society Criteria for HLH) if they had ferritin $\geq 10,000$ $\mu\text{g/L}$, or sCD25 and NK cell function tests had been requested. Patients were also included if they had a bone marrow biopsy result that suggested the presence of haemophagocytosis. For patients with multiple ferritin results of $\geq 10,000$ $\mu\text{g/L}$, the highest one was recorded for analysis, except for the subgroup analysis (n=59) described below.)

Cases of bone marrow haemophagocytosis were identified by first searching for “phagocyt” in the “histiocyte comment” and “summary” of bone marrow reports from the same study period; this search term captures comments “haemophagocytosis”, “actively phagocytic”, “phagocytosis of”, and “erythrophagocytosis”; all of which can be used to describe haemophagocytosis. Whenever available, bone marrow samples

identified from the final compiled patient list were retrieved and independently reviewed by another haematologist (DT). Bone marrow haemophagocytosis was considered to be present if reported by either the original reporting haematologist or the independent reviewer.

Fifty-nine bone marrow samples from the available marrow list were scored by the independent reviewing haematologist for haemophagocytosis. Only cases that showed macrophages engulfing intact cells were counted as having evidence of haemophagocytosis. Haemophagocytic cells were quantified as 0, 1-4, and ≥ 5 per slide. Correlation between the extent of bone marrow haemophagocytosis and the number of HLH criteria patient satisfied as well as correlation between the extent of bone marrow haemophagocytosis and patient's highest ferritin levels were assessed. If highest ferritin was recorded as a semi-quantitative data, then the second highest quantitative ferritin level were used instead.

Those with insufficient medical information documented near the time of abnormal results were excluded from the study. If the patients from the list satisfied ≥ 4 out of 8 criteria then their clinical course was evaluated regarding likely triggers, underlying co-morbidity, treatment, and outcome. Survival status was determined in July 2017 based on information available in existing medical records.

Statistics

GraphPad Prism 7 was used to generate Kaplan Meier Survival Analysis and Log-Rank Test to compare survival of patients. For significance testing, we set $\alpha=0.05$.

Results

Case ascertainment

Four patients were identified with possible HLH based on ICD code assigned at hospital discharge, two of whom were adults at the time of admission (**Fig. 1**). None of these patients were thought to have HLH or MAS by the treating team, and we determined that none satisfied ≥ 4 diagnostic criteria for HLH. We concluded that these were coding errors and the patients were excluded from further analysis.

Next, we ascertained cases according to diagnostic criteria. Ferritin levels were reported as quantitative (n=105) and semi-quantitative (n=56) data over the study period (**Table 1**). A total of 131 patients had ferritin levels $\geq 10,000$ $\mu\text{g/L}$. An additional 30 patients had ferritin levels of >2000 $\mu\text{g/L}$ without further quantification, results which are ambiguous with regard to our study criteria, we nevertheless elected to include these patients. We noted from review of the clinical records that 31 of 161 patients did not have hospital admission at our institution near the time of their significant hyperferritinaemia. As discussed below, assessment for HLH criteria was often incomplete. Only 38 out of 161 patients had bone marrow biopsy, 48 patients had serum triglycerides measured, 87 patients had serum fibrinogen measured, and 118 patients had spleen size estimated either by physical exam or by imaging.

Of the 161 patients with hyperferritinaemia, 155 patients were adults (≥ 18 -year-old) at the time of the ferritin test (**Fig. 1**). Of the adult patients with hyperferritinaemia, 21 satisfied ≥ 5 HLH diagnostic criteria, 19 satisfied 4 criteria, and 115 satisfied ≤ 3 criteria. Of the 21 patients who satisfied ≥ 5 HLH diagnostic criteria, 14 received a diagnosis of HLH by the treating team during the course of their illness. Of the 19 patients who satisfied 4 criteria, only 3 patients had received a diagnosis of “likely HLH” during the course of their illness. Of the 115 patients who satisfy ≤ 3 criteria, only 1 patient was diagnosed with probable HLH.

High serum ferritin levels were associated with increased likelihood of satisfying ≥ 5 HLH diagnostic criteria (**Table 2**). 28% of patients with ferritin levels $> 40,000$ $\mu\text{g/L}$ satisfied ≥ 5 HLH diagnostic criteria compared to 11% of patients with ferritin levels of 10,000-40,000 $\mu\text{g/L}$. By contrast, most patients who satisfied ≤ 3 criteria had low ferritin levels. Of note though, even amongst patients with a high ferritin level ($> 40,000$ $\mu\text{g/L}$), the majority (61%) satisfied ≤ 3 HLH diagnostic criteria.

Of the 36 adult patients who had hyperferritinaemia of $> 40,000$ $\mu\text{g/L}$, 22 patients satisfied ≤ 3 HLH diagnostic criteria (**Table 2**). Sepsis (4/18, 22%), acute hepatitis (9/18, 50%), flare-up of rheumatological disease (3/18, 17%), and multi-organ failure secondary to other causes (2/18, 11%) accounted for cases of hyperferritinaemia amongst those who satisfied ≤ 3 HLH diagnostic criteria. Incomplete documentation precluded analysis of causes of hyperferritinaemia in four of the 22 patients.

Over the study period, assessment of NK cell function was requested on only four patients, suggesting remarkable under-utilisation of this test. Three of these four patients were adults when NK cell cytotoxicity was tested. Review of these results revealed that one patient had reduced NK cell cytotoxicity and reduced NK cell perforin expression, and this patient had a ferritin level of $>40,000 \mu\text{g/L}$ and satisfied five HLH criteria. Another patient had reduced perforin expression and poor NK cell function, but this result was ambiguous because of NK cell lymphopenia. This patient had a high ferritin level of $43005 \mu\text{g/L}$ and satisfied five HLH criteria. One patient had normal perforin expression, and this patient had a ferritin level of $4142 \mu\text{g/L}$ and satisfied only two HLH criteria. In this very limited sample, reduced NK cell function and perforin expression appears to distinguish between those who have HLH from those who do not. Two of the three adult patients from NK function list satisfied ≥ 4 criteria, both met the hyperferritinaemia criterion for inclusion.

Serum sCD25 was ordered for only five patients during the entire study period. Of these five patients, four were adults. Three adult patients had elevated sCD25 level (median 20419 pg/mL ; range $3143\text{-}45479 \text{ pg/mL}$; normal range was $186\text{-}2678 \text{ pg/mL}$), and all of them had elevated ferritin levels (range $10029 - >99,999 \mu\text{g/L}$). Two of them satisfied six criteria, the other one satisfied two criteria. One of the four adult patients had normal sCD25 level (1841 pg/mL), and this patient had a highest ferritin level of $46886 \mu\text{g/L}$, and satisfied six HLH criteria. Based on this limited sample, serum sCD25 on its own may not be able to differentiate those who have HLH from those who do not.

Over the study period, 43 patients had bone marrow biopsy evidence of haemophagocytosis. All of them were over the age of 18 years at the time of their biopsy. Of these, 24 (56%) patients satisfied ≥ 4 HLH diagnostic criteria (13 satisfied ≥ 5 criteria and 11 satisfied 4 criteria). Of the 24 patients, 18 had a ferritin level $>10,000$ $\mu\text{g/L}$. One patient did not have a ferritin level assessed near the time of their bone marrow biopsy. The remaining five patients had ferritin levels ranging from 873 – 8217 $\mu\text{g/L}$. Focusing on the 13 patients who satisfied ≥ 5 criteria, two of them had ferritin levels $<10,000$ $\mu\text{g/L}$ (1163 $\mu\text{g/L}$ and 7320 $\mu\text{g/L}$). Overall, six additional patients who had haemophagocytosis satisfied ≥ 4 HLH diagnostic criteria, however, one patient was excluded from further analysis due to lack of sufficient clinical information recorded near the time of bone marrow biopsy. In addition, one adult patient who satisfied ≤ 3 HLH criteria was deemed to have HLH.

We also evaluated bone marrow haemophagocytosis semi-quantitatively. Fifty-nine bone marrow samples were reviewed and scored for severity of haemophagocytosis. There was no correlation between extent of bone marrow haemophagocytosis and the number of diagnostic criteria met (**Table 4**). Interestingly increased bone marrow haemophagocytosis did not correlate with higher ferritin level either (**Table 5**).

Survival

A total of 23 adult patients (14 male, 9 female) satisfied ≥ 5 HLH diagnostic criteria (**Table 3**). As of July 2017, 7 out of 23 (30%) patients were alive. Median age at disease onset (based on hyperferritinaemia or bone marrow abnormality) was 55 years (range

18-81 years). Twenty patients had a highest ferritin of $\geq 10,000$ $\mu\text{g/L}$. The remainder had highest recorded ferritin level reported as 1163 $\mu\text{g/L}$, 7320 $\mu\text{g/L}$, and >2000 $\mu\text{g/L}$. Sixteen patients who satisfied ≥ 5 HLH diagnostic criteria were thought to have HLH by the treating team.

Of these 23 patients, 11 had underlying malignant haematological disorder, four had significant infection in setting of underlying autoimmune or inflammatory disease on immunosuppressants, one had primary immunodeficiency, three had severe infection, and four were considered to have had primary HLH. Of the 11 with malignant haematological disorder, four had T cell lymphoma, three had B cell lymphoma, one had anaplastic large cell lymphoma, one had acute lymphoblastic leukaemia (ALL), one had myelodysplastic syndrome, the last had an undefined lymphoproliferative disease.

Of the four patients who developed significant infection in the setting of underlying autoimmune or inflammatory disease, one had primary varicella zoster virus (VZV) infection whilst on systemic immunosuppression for eczema, one had primary VZV infection on background of systemic lupus erythematosus (SLE), one had culture-negative sepsis on background of SLE, and another had culture-negative sepsis on background of primary biliary cholangitis (PBC). Of the three patients with severe infection, one had both active human immunodeficiency virus (HIV) and hepatitis B (HBV) infection, another had primary Epstein-Barr virus (EBV) infection, and the third had culture-negative sepsis.

Based on the ferritin results for the 23 adult patients who satisfied ≥ 5 HLH diagnostic criteria, the sensitivity of ferritin $\geq 10,000$ $\mu\text{g/L}$ (for HLH) was between 87% (20/23, if we exclude ferritin " >2000 " samples) and 91% (21/23, if we include ferritin " >2000 " samples). False negatives were cases that satisfied ≥ 5 HLH but did not meet ferritin $\geq 10,000$ $\mu\text{g/L}$ criteria. Based on the bone marrow results of these 23 patients, the sensitivity of bone marrow haemophagocytosis for HLH was 78% (18/23). False negative bone marrow results included cases that satisfied ≥ 5 HLH but did not have bone marrow haemophagocytosis as determined by both the original reporting haematologist and an independent review.

Survival of adult patients who satisfied ≥ 5 HLH diagnostic criteria according to the causes of their diseases is shown in **Figure 2**. Haematological malignancies and non-malignant haematological diseases carry poorer prognoses, each with median survival of approximately one month from diagnosis. Patients with primary HLH, severe infection, or significant infection in the setting of autoimmune disease survived for much longer.

A total of 23 adult patients satisfied four HLH diagnostic criteria. It is unclear whether they represent possible HLH in view of the often incomplete assessment. Of the adult onset patients, 14 were male, and 9 were female. The median onset of disease for the adult patient was 61 (range 21-89). Of the adult-onset patients, 18 had highest ferritin levels $\geq 10,000$ $\mu\text{g/L}$. The remainder had highest ferritin level of 873 $\mu\text{g/L}$, 2730 $\mu\text{g/L}$, 3501 $\mu\text{g/L}$, 8217 $\mu\text{g/L}$, and >2000 $\mu\text{g/L}$. Of the adult onset patients, 15 had underlying haematological malignancies, one had non-malignant haematological disorders, three

had necrotizing organ inflammation, one had sepsis whilst on immunosuppression for autoimmune disease, and three had unclear causes. Of the adult-onset patients, five were thought to have HLH by the treating team.

Interestingly, the survival of patients who satisfied four HLH diagnostic criteria did not differ significantly from patients who satisfied ≥ 5 criteria. (Fig. 3). The median survival for those who satisfied ≥ 5 criteria was three months, and for those who satisfied four criteria was two months. Log-rank test was performed, and there was no statistically significant difference was detected between the two groups (p value = 0.47). These findings suggest that a substantial subset of those satisfying only four HLH criteria did in fact have HLH.

Discussion

In this report of a single centre experience of the diagnostic approach to HLH, we observed infrequent use of the tests that might ascertain cases according to established diagnostic criteria. Assessment of 8 diagnostic criteria was rarely completed by the treating team. In particular, sCD25 and NK cytotoxicity assays were seldom ordered. For NK function, this may in part be contributed by the fastidious nature of the assay that requires complex co-ordination from the point of blood collection to transport to the reference laboratory to ensure validity of the test. In addition, the test is most helpful if performed before commencement of corticosteroid treatment (which can suppress NK function). These constraints do not explain why soluble CD25 is not measured more frequently. In addition, clinician awareness of HLH and the approach to its diagnosis is

often lacking, and a likely explanation for under investigation of patients suspected of having HLH is that clinicians are simply unfamiliar with the diagnostic criteria. Indeed, we observed that even when ≥ 4 criteria were met, the diagnosis of HLH was not considered by the treating team. Not surprisingly, therefore, when patients satisfied ≤ 3 criteria, the diagnosis of HLH was rarely entertained by the treating team. We acknowledge that results from a single centre might not be typical of approaches elsewhere, it is possible that our findings are more generalizable, and therefore we highlight this diagnostic shortcoming.

In our cohort, bone marrow haemophagocytosis carried a sensitivity of 78% for adult-onset HLH. This is a little lower than the 88-97% reported in studies from USA and China.^{9, 10} Of the patients who had bone marrow evidence of haemophagocytosis in our cohort, only 30% satisfied diagnostic criteria for HLH. This is similar to the 29% reported in Lim *et al*'s Korean cohort.¹¹ In our subgroup analysis, we did not identify a correlation between extent of bone marrow haemophagocytosis and the number of diagnostic criteria patients satisfied, or with the highest ferritin level recorded. These findings are in keeping with those reported by Ho *et al*,¹² and highlight that haemophagocytosis alone is not sufficient to make the diagnosis. This may be explained in part by the subjective nature of bone marrow assessment in absence of defined morphologic guidelines. Other conditions that are associated with bone marrow haemophagocytosis include malignant haematological disease, infectious diseases, and some autoimmune disease.¹¹

We found that hyperferritinaemia $\geq 10,000$ $\mu\text{g/L}$ carried a diagnostic sensitivity of 87-91% for adult-onset HLH. The high sensitivity of severe hyperferritinaemia means that accurate reporting of very high ferritin is important. While accurate quantification of serum ferritin necessitates serum dilution and is therefore a burden on the diagnostic laboratory, this appears warranted given the importance of a timely and accurate diagnosis of HLH. Serum ferritin $> 10,000 \mu\text{g/L}$ should prompt further investigation for HLH. We note as well that Parikh *et al* reported that the sensitivity of ferritin in detecting adult-onset HLH declined from 98% to 64% when the threshold for hyperferritinaemia was increased from 500 $\mu\text{g/L}$ to 3000 $\mu\text{g/L}$.⁹ It is possible that by reducing the ferritin threshold in our study, more cases of adult-onset HLH could be detected and therefore increasing the assay sensitivity.

Even though hyperferritinaemia is highly sensitive, it is associated with a high false positive rate. In our study, of those with hyperferritinaemia $\geq 10,000$ $\mu\text{g/L}$, only between 13% (21/161, including patients with highest ferritin of $> 2,000$ $\mu\text{g/L}$) and 15% (20/131, excluding patients with highest ferritin of $> 2,000$ $\mu\text{g/L}$) satisfied HLH diagnostic criteria. This is higher than reported by Sackett *et al* (6%) in a shorter study (2007-2012) that included fewer patients with serum ferritin levels of $\geq 10,000$ $\mu\text{g/L}$.¹³ Other medical conditions that are associated with hyperferritinaemia include chronic transfusion, liver disease, and hematologic malignancy.^{13, 14}

Improving awareness of the condition, and the application of current diagnostic criteria should improve the diagnostic rate for HLH, but more sensitive and specific markers for adult-onset HLH are also required. Assessing glycosylated ferritin level in addition to

ferritin may be of diagnostic value, as there is evidence to suggest the combination of high ferritin level and low percentage of glycosylated ferritin (such as <20%) may be specific for adult-onset HLH.^{15, 16} Neopterin is a secreted product of activated macrophages and dendritic cells. In children, neopterin level of >38.9 nmol/L was found to be 70% sensitive and 95% specific for a diagnosis of HLH.¹⁷ However, its sensitivity and specificity has not been formally evaluated in the adult-onset HLH group. The presence of haemophagocytosis in bone marrow is often subjective, especially so in the absence of published guidelines for morphologic diagnosis.

Since advanced biomarkers for HLH may not be readily available in all centres, other approaches to improving diagnostic sensitivity have been explored. The H score was designed in 2014 by Farget et al and validated for primary and secondary HLH in both paediatric and adult populations.^{18, 19} The H score is derived from nine clinical, biochemical, and histological variables, and a score of 169 was found to have a sensitivity of 93% and a specificity of 86% for adult-onset secondary HLH.¹⁹ Reduction of the H score has been shown to improve sensitivity at the cost of reduced specificity when compared with diagnosis based on satisfying 4 or 5 HLH-2004 criteria.¹⁸

A consistent molecular diagnosis with HLH alone is an alternative way to meet the HLH-2004 diagnostic criteria of HLH. In our cohort of patients who satisfied ≥ 4 diagnostic criteria, only one patient was referred for *PRF1* mutation testing (by Sanger sequencing). In two large case series of adult-onset HLH from Chinese population, 7-14% patients were found to have mutations in HLH related genes (*PRF1*, *STX11*, *SH2D1A*, *UNC13D*, *STXBP2*) after panel-based sequencing of known HLH-related genes (exons and

introns).^{20, 21} In a small Italian series of adult-onset primary HLH, mutations in *PRF1*, *SH2D1A*, *UNC13D*, and *STXBP2* were found.⁽²²⁾ Whole exome and whole genome sequencing is a suitable approach for genetically heterogeneous diseases such as HLH, but a systemic assessment of this approach for adult-onset HLH is yet to be reported.

In summary, our report highlights the need for a consistent approach to the diagnosis and management of adult-onset HLH in order to improve outcomes for this rare and lethal condition. We demonstrate the sensitivity of hyperferritinaemia and haemophagocytosis on bone marrow biopsy in diagnosis of adult-onset HLH in an Australian tertiary hospital. Further research into better diagnostic tool may not only fast-track diagnosis but also shed light to the underlying disease pathogenesis.

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Table 1. Number of patients who satisfied ferritin level criteria ($\geq 10,000$ $\mu\text{g/L}$)

Ferritin level ($\mu\text{g/L}$)	N=
>99,999*	4
90,000-100,000	3
>90,000*	1
80,000 – 90,000	0
70,000 – 80,000	3
60,000 – 70,000	0
50,000 – 60,000	2
40,000 – 50,000	4
>40,000*	21
30,000 – 40,000	8
20,000 – 30,000	19
10,000 – 20,000	66
>2000*	30
Total N = 161	

N= number of patients identified

* reported semi-quantitatively without further quantification

Table 2. Correlation between ferritin level and the number of criteria satisfied by adult patients.

Ferritin level ($\mu\text{g/L}$)	≥ 5 criteria	4 criteria	≤ 3 criteria	N=
>40,000	10 (28%)	4 (11%)	22 (61%)	36 (100%)
10,000 – 40,000	10 (11%)	14 (15%)	67 (74%)	91 (100%)
>2000*	1 (3.5%)	1 (3.5%)	26 (93%)	28 (100%)

N= number of patients identified for each ferritin level

* reported semi-quantitatively without further quantification

Table 3. Summary of characteristics of patients who satisfied ≥ 5 HLH criteria

	Age of onset	Sex	Aetiology	Fever	Sple-nome-galy	Bi-cyto-penia	High TG/ Low fibrin -ogen	High Ferritin	BM haemo-phago-cytosis	NK dys-func-tion	High sCD25	HLH dx by home team	HLH specific tx
1	58	M	T cell lymphoma	+	-	+	+	>99999	+	N/A	+	Yes	HLH-94 followed by CHOEP chemotherapy
2	86	M	Anaplastic large cell lymphoma	+	-	+	+	>40000	+	N/A	N/A	Yes	Methylprednisolone
3	37	F	T cell lymphoma	+	+	+	-	97838	+	N/A	N/A	Yes	Alemtuzumab and dexamethasone
4	55	F	T cell lymphoma	+	-	+	+	35824	+	N/A	N/A	Yes	Cyclosporin
5	65	M	Lymphoproliferative disorder	+	+	+	+	25017	+	N/A	N/A	No	N/A
6	43	F	ALL	+	+	+	-	14625	+	N/A	N/A	No	N/A
7	75	M	Lymphoplasmacytic lymphoma	+	+	+	+	18262	-	N/A	N/A	No	N/A
8	66	F	Myelodysplastic/ myeloproliferative overlap syndrome	+	+	+	+	17383	-	N/A	N/A	No	N/A
9	78	M	B cell lymphoma	+	+	+	+	15990	-	N/A	N/A	Yes	Nil
10	76	F	T cell lymphoma	+	+	+	+	>40000	-	N/A	N/A	No	N/A
11	64	M	Hodgkin's lymphoma	+	+	+	-	>2000	+	N/A	N/A	No	N/A
12	39	M	Primary HLH	+	N/A	-	+	>40000	+	+	N/A	Yes	HLH-94
13	44	F	Primary HLH	+	-	+	+	>40000	+	N/A	N/A	Yes	Modified HLH-94*
14	80	M	Primary HLH	+	+	+	+	29051	+	-	+	Yes	Modified HLH-94*
15	25	M	Primary EBV	+	+	-	+	71908	+	N/A	N/A	Yes	Nil acutely; SCT 3 years after for HLH relapse
16	62	M	Sepsis (pseudomonas)	+	-	+	+	58375	+	N/A	N/A	No	N/A
17	51	M	Primary VZV and HHV-	+	-	+	+	46886	+	N/A	N/A	Yes	Dexamethasone,

			6 re-activation b/g atopic dermatitis on immunosuppressant										IVIg, plasma exchange, and acyclovir; followed by azathioprine
18	38	F	Primary VZV b/g SLE	+	+	-	+	26196	+	N/A	N/A	Yes	Prednisolone, IVIG, and acyclovir
19	41	F	Sepsis b/g SLE	+	-	+	+	12828	+	N/A	N/A	Yes	Prednisolone
20	39	M	Sepsis b/g PBC	+	+	-	+	14928	+	N/A	N/A	Yes	Prednisolone
21	41	F	Sepsis b/g Good's syndrome	+	-	+	+	43005	+	-	N/A	Yes	Prednisolone and IVIG
22	52	M	HIV + HBV	+	+	+	-	7320	+	N/A	N/A	Yes	Dexamethasone
23	58	M	Primary HLH	+	+	-	+	1163	+	N/A	N/A	Yes	Dexamethasone, cyclosporine, and vincristine; followed by tocilizumab

b/g: on background of; TG: triglyceride; BM: bone marrow; +: satisfied criteria; -: did not satisfied criteria, N/A: not assessed or not applicable, tx: treatment; modified HLH-94*: used similar regime to HLH-94 with modification; SCT: stem cell transplant; IVIG: intravenous immunoglobulin

Table 4. Correlation between the degree of bone marrow haemophagocytosis and total number of criteria patient satisfied

Number of criteria patient satisfied	≤3	4	≥5
HPC 0 per slide	11	7	7
HPC 1-4 per slide	6	4	6
HPC ≥5 per slide	6	6	6

HPC: haemophagocytic cell

Table 5. Correlation between the degree of bone marrow haemophagocytosis and patients' highest ferritin levels

Highest ferritin level	<500µg/L	500-100,00µg/L	>10,000µg/L
HPC 0 per slide	2	5	15
HPC 1-4 per slide	1	5	8
HPC ≥5 per slide	2	4	9

Figure 1. Study design to identify patients with adult-onset HLH.

238x144mm (96 x 96 DPI)

Figure 2. Comparing survival of adult patients who satisfied ≥ 5 vs 4 HLH criteria.

205x100mm (96 x 96 DPI)

Figure 3. Comparing survival of adult patients who satisfied 5+ criteria by causes of disease.

259x121mm (144 x 144 DPI)