Haemophagocytic lymphohistiocytosis (HLH) is a condition involving cytokine overproduction by defective cytotoxic T lymphocytes and natural killer (NK) cells. Massive amounts of proinflammatory cytokines (interleukins 1, 6 and 10, granulocyte macrophage colony stimulating factor, tumour necrosis factor-α and interferon-γ (IFN-γ)) released by CTLs result in increased production of non-neoplastic interleukins 1, 6 and 10, granulocyte macrophage colony stimulating factor and IFN-γ.

HLH may be classified as primary/genetic or secondary/acquired (Table 1). In genetic HLH, a mutation occurs in one of the proteins responsible for intracellular vesicle docking and release of preformed proapoptotic granzyme and perforin. This in turn reduces the ability of CTLs and NK cells to induce apoptosis of antigen-presenting cells and clonally expanded T lymphocytes. In acquired HLH, the exact cause of depressed CTL and NK cell activity is less well understood, but it is strongly associated with specific viral infections, malignancies and autoimmune disorders. It is postulated that patients who develop acquired HLH may in fact have an underlying (as yet unidentified) genetic predisposition.

Genetic HLH arises in childhood, usually before 1 year of age, but there are reports of adults presenting with primary HLH at advanced ages. As it is due to a genetic mutation, the only chance of cure is stem cell transplantation.

Acquired HLH can occur at any age and is treated with combination chemotherapy via the HLH-94 trial protocol, along with treatment for the suspected trigger (e.g. infection). In cases with a clear trigger for acquired HLH (e.g. cytomegalovirus (CMV), HIV), reports of complete cure (using immunoglobulins or antiretrovirals) without the need for additional chemotherapy have been described.

Patients who are diagnosed with acquired HLH that does not respond to the HLH-94 chemotherapy regimen or relapse post-chemotherapy are referred for stem cell transplantation.

Much of our knowledge about HLH is derived from Swedish paediatric studies in the context of primary HLH, where an incidence of 0.12/100 000/year has been estimated. The exact incidence of acquired HLH in adults is unknown, although a single-institution retrospective analysis of malignancy-associated acquired HLH puts the estimate at 0.36/100 000/year.

The diagnostic criteria for HLH are published by the Histiocyte Society (http://www.histiocytesociety.org) and require either a confirmed molecular diagnosis of genetic mutations known to be associated with HLH or the presence of at least 5/8 clinical and laboratory criteria. These include:

- prolonged fever
- splenomegaly
- haemophagocytosis (in bone marrow, lymph node or spleen)
- cytopenias
HLH can mimic many other conditions in its early stages (Table 2). It may therefore be underdiagnosed in patients with infection or malignancy who present with systemic inflammatory response syndrome (SIRS) and acute multiorgan dysfunction. A high index of suspicion is required to promptly initiate therapy in these patients.

Because awareness of acquired HLH is limited, it typically has a high mortality rate (50 - 100%), partly as a result of delayed diagnosis and treatment. Recent studies suggest that acquired HLH is an under-recognised cause of death in adult intensive care units (ICUs). Patients usually present with features of SIRS (including pyrexia of unknown origin, tachycardia and leucopenia) and disseminated intravascular coagulation (DIC), organomegaly (specifically splenomegaly with or without hepatomegaly), and may additionally exhibit hepatic dysfunction, neurological deficits or septic features. As not all diagnostic features may be present initially, it is essential to monitor patients clinically and biochemically at regular intervals. In the early stages, there may be mild manifestations (e.g. skin rashes) that resolve spontaneously with subsequent exacerbations.

Importantly, haemophagocytosis is not specific to HLH and can be demonstrated in various non-HLH conditions such as post-blood transfusion, haemolysis, myelodysplasia/bone marrow failure or sepsis. Moreover, haemophagocytosis may not be seen in up to 20% of initial bone marrow biopsies and therefore, despite the nomenclature, can neither be diagnosed nor excluded solely on the basis of presence or absence of haemophagocytosis.

We describe five cases of acquired HLH that were diagnosed over the course of 5 months (May - September 2013) in private hospitals in the greater Johannesburg area, South Africa (SA), with the aim of raising awareness and increasing the diagnostic pick-up rate of acquired HLH in adult patients admitted to wards and ICUs with multiorgan dysfunction and/or presumed sepsis/SIRS. Although only three patients in this series were HIV-positive, the high burden of HIV in SA may make this condition more common, potentially triggered by coexisting HIV-associated infections and malignancies.

All patients were diagnosed with acquired HLH following bone marrow aspiration and trephine (BMAT). Clinical information (viz. presenting signs and symptoms, temperature trends, organomegaly) was obtained either from the bedside at time of BMAT or alternatively via communication with the treating physician. All laboratory data were accessed on the password-protected Meditech software system at Lancet Laboratories.

The clinical and laboratory details of the cases are shown in Table 3. Four cases fulfilled the diagnostic criteria for HLH. These include at least 5/8 criteria (listed as B1 - B8). No genetic mutational analyses were performed (A). Individual results that fit the HLH diagnostic criteria are highlighted in bold. Case 2 fulfilled only four criteria, but was included in the case series in view of her markedly raised ferritin level (24 370 μg/L), hepatomegaly and active haemophagocytosis. (Had NK cell activity and/or CD25 assays been available, these may have been able to confirm the diagnosis conclusively.)

All patients were young adults. Cases 2 and 4 tested positive for HIV prior to admission and were receiving treatment. During admission, a drug-resistance polymerase chain reaction (PCR) screen performed on case 2 showed resistance to efavirenz. Case 1, who was initially HIV-negative according to an enzyme-linked immunosorbent assay (ELISA) and p24 antigen assay, seroconverted during the course of treatment.

Cases 1 and 2 were diagnosed with diffuse large B-cell lymphoma at the time of HLH diagnosis. Neither had undergone chemotherapy for lymphoma prior to HLH diagnosis. Case 2 additionally had a prior history of high-grade ductal carcinoma. Case 4 had been treated for Kaposis’s sarcoma (KS) prior to HLH diagnosis.

All patients had prolonged fever prior to the diagnosis of HLH. This feature is consistent among HLH patients and is due to CTL/NK-mediated hypercytokinaemia rather than to specific infectious agents. Fever generally do not resolve despite the use of empiric antibiotics.

Splenomegaly was assessed either clinically or radiologically, absence of splenomegaly in cases 2 and 4 being confirmed on radiological imaging. Splenomegaly is a fairly consistent feature in HLH and is due to organ infiltration by activated histiocytes and
Hepatomegaly may similarly develop and commonly results in deranged liver enzymes secondary to hepatocyte damage. Organomegaly was shown to be present in 90% of HLH patients in a recent Indian case series. All patients exhibited cytopenias during their stay, although cell counts often fluctuated markedly, most commonly following transfusion of blood products. As a result, the diagnostic bi-/pancytopenia may not be present following blood/platelet transfusion, a fact to be borne in mind when working up a patient for suspected HLH. Importantly, underlying neutropenia may be masked if recombinant growth factors (e.g. filgrastim) have recently been administered.

All the patients demonstrated haemophagocytosis in the bone marrow, probably because all were at an advanced stage of illness at the time of BMAT and their haemophagocytosis was well established. The presence of haemophagocytosis signalled the possibility of HLH and prompted urgent communication with the treating physicians and additional focused laboratory investigations including ferritin, fibrinogen and triglyceride levels.

Three patients had raised triglycerides above the diagnostic 3 mM level, although the remaining two showed levels closely approaching this (2.91 mM and 2.95 mM). Fasting hypertriglyceridaemia is seen in ~70% of HLH patients and is thought to be the result of cytokine-mediated lipoprotein lipase inhibition.

Ferritin and fibrinogen are liver-derived positive acute-phase reactants. In the normal inflammatory response, levels of both should increase. However, markedly raised ferritin levels in HLH denote excessive production by activated histiocytes and possibly release from damaged hepatocytes.

**Table 2. Mechanisms involved and differential diagnoses of typical clinical and laboratory findings in HLH**

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Mechanism</th>
<th>Differential diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytopenia/s</td>
<td>Haemophagocytosis via activated histiocytes</td>
<td>Bone marrow hypoplasia/ failure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bone marrow infiltration (malignant, infective)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drug-induced myelotoxicity (e.g. HAART, chemotherapy)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sepsis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ITP/TPP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sequestration (e.g. splenomegaly)</td>
</tr>
<tr>
<td>Liver dysfunction</td>
<td>Infiltration by activated histiocytes</td>
<td>Viral hepatitis</td>
</tr>
<tr>
<td></td>
<td>Hepatocyte damage</td>
<td>Drug-induced hepatitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Warfarin toxicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DIC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sepsis</td>
</tr>
<tr>
<td>Fever</td>
<td>Pro-inflammatory cytokine release by CTL/NK cells</td>
<td>Infection</td>
</tr>
<tr>
<td>Neurological deficit/s</td>
<td>Demyelination</td>
<td>Infections (meningitis, encephalitis)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CVA, space-occupying lesion</td>
</tr>
<tr>
<td>Hyperferritinaemia</td>
<td>Released from activated histiocytes</td>
<td>Sepsis</td>
</tr>
<tr>
<td></td>
<td>? Released from damaged hepatocytes</td>
<td>Anaemia of chronic disorders</td>
</tr>
<tr>
<td></td>
<td>Ferritin receptor down-regulation</td>
<td>Iron overload disorders</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SLE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fulminant hepatic failure</td>
</tr>
<tr>
<td>Hypertriglyceridaemia</td>
<td>Liver infiltration by histiocytes</td>
<td>Dyslipidaemia</td>
</tr>
<tr>
<td></td>
<td>Decreased levels of lipoprotein lipase</td>
<td>Diabetes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nephrotic syndrome</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drugs</td>
</tr>
<tr>
<td>Hypofibrinogenaemia</td>
<td>Liver infiltration</td>
<td>DIC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver failure</td>
</tr>
<tr>
<td>Hepatomegaly/splenomegaly</td>
<td>Organ infiltration by activated histiocytes</td>
<td>EPTB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Malignancies (e.g. CML, hepatic metastases)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Infections (e.g. malaria)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemolytic states</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Extramedullary haematopoiesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Storage disorders</td>
</tr>
<tr>
<td>Cutaneous manifestations</td>
<td>Histiocytic and lymphocytic infiltration</td>
<td>Eczema</td>
</tr>
<tr>
<td>(commonly panniculitis and/or purpura)</td>
<td></td>
<td>Erythema nodosum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Panniculitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drug-related</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kawasaki disease</td>
</tr>
</tbody>
</table>

HLH = haemophagocytic lymphohistiocytosis; HAART = highly active antiretroviral therapy; ITP = idiopathic thrombocytopenic purpura; TTP = thrombotic thrombocytopenic purpura; DIC = disseminated intravascular coagulation; CTL = cytotoxic T-lymphocyte; NK = natural killer; CVA = cerebrovascular accident; SLE = systemic lupus erythematosus; EPTB = extrapulmonary tuberculosis; CML = chronic myeloid leukaemia.

*Adapted from Lehmberg and Ehl, Usmani et al., Jordan et al., Okabe et al., Raschke and Garcia-Ony and Morrell et al.*
Ferritin levels were markedly raised in all cases. Though the diagnostic threshold is 500 µg/l, reports show that levels >3 000 µg/l should raise strong suspicion and initiate prompt investigation for HLH. Levels >10 000 µg/l have been shown to be >90% specific for HLH, though they may also be associated with fulminant hepatic failure. Ferritin is a useful, rapid and relatively cost-effective parameter to measure, especially in resource-limited settings.

### Table 3. Case series of patients diagnosed with acquired HLH*

<table>
<thead>
<tr>
<th>Age (yrs), gender</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>(32, male)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(34, female)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(25, female)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(47, male)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(29, female)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### HLH diagnostic criteria

A. Molecular diagnosis for HLH-associated genetic mutations

| ND | ND | ND | ND | ND |

B1. Fever

Present

Present

Present

Present

Present

B2. Splenomegaly

Present (absent (mild hepatomegaly))

Present

Present

Present

Present

B3. Anaemia (Hb ≤ 9 g/dl)

15.4

7.3

7.7

8.8

Present

B. Absolute neutropenia (≤1×10⁹/l)

0.24

0.1

8.62

16.8

8.62

B. Thrombocytopenia (≤100×10⁹/l)

23

30

49

9

10

B4. Haemophagocytosis present in BM, spleen or lymph node

Present (BM)

Present (BM)

Present (BM and lymph node)

Present (BM and lymph node)

Present (BM and lymph node)

B5. Fasting hypertriglyceridaemia (≥3 mmol/l) and/or hypofibrinogenaemia (≤1.5 g/l)

4.22

2.91

4.20

2.95

7.79

B6. Hyperferritinaemia (≥500 µg/l)

11 427

24 370

19 115

4 072

147 952

B7. Increased soluble CD25 (≥2 400 U/ml)

ND

ND

ND

ND

ND

B8. Low/absent NK cell activity

ND

ND

ND

ND

ND

**Additional investigations**

<table>
<thead>
<tr>
<th>HIV status</th>
<th>Positive</th>
<th>Positive</th>
<th>Negative</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herpesvirus (EBV, CMV, HHV-8)</td>
<td>ND</td>
<td>EBV-positve on trephine</td>
<td>Negative</td>
<td>EBV serology-negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Hypofibrinogenaemia</td>
<td>ND</td>
<td>ND</td>
<td>0.94</td>
<td>3.70</td>
<td>0.6</td>
</tr>
<tr>
<td>TB</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Fungal</td>
<td>ND</td>
<td>ND</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Blood cultures</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>CoNS</td>
<td>Negative</td>
</tr>
<tr>
<td>PCR</td>
<td>ND</td>
<td>Negative for P. jiroveci</td>
<td>Negative for CMV</td>
<td>ND</td>
<td>Negative for H1N1, influenza A and B</td>
</tr>
<tr>
<td>Autoimmune screen</td>
<td>ND</td>
<td>ND</td>
<td>Negative for CTD</td>
<td>ND</td>
<td>Negative for c-ANCA, anti-glomerular base</td>
</tr>
</tbody>
</table>

### Associated malignancy

Yes

Yes

No

Yes

No

### Likely trigger of HLH

Diffuse large B-cell lymphoma

Uncertain infection

Sepsis

? HIV

? Autoimmune

### Clinical outcome

Alive

Died

Died

Died

Alive

---

HLH = haemophagocytic lymphohistiocytosis; ND = not done; Hb = haemoglobin; BM = bone marrow; NK = natural killer; HIV = human immunodeficiency virus; EBV = Epstein-Barr virus; CMV = cytomegalovirus; HHV-8 = human herpesvirus-8; EBNA = Epstein-Barr virus nuclear antigen 1; KS = Kaposi’s sarcoma; TB = tuberculosis; CoNS = coagulase-negative Staphylococcus; PCR = polymerase chain reaction; CTD = connective tissue disease; c-ANCA = cytoplasmic anti-neutrophil cytoplasmic antibody.

*Individual results that fit the HLH diagnostic criteria are highlighted in bold.

†Total white cell count. A valid differential count could not be performed because there were too few leucocytes.

‡Determined by in situ hybridisation.
Fibrinogen levels were measured in cases 3 - 5 only (as part of DIC screening) with cases 3 and 5 showing diagnostically low levels.

Neither NK cell activity nor CD25 assays were performed, as neither assay is widely available locally. Owing to technical aspects of the assays, if available, and delay in obtaining results, it is recommended that blood be taken at the earliest possible opportunity during work-up for suspected HLH. For all the patients in this series, NK cell or CD25 assays may have been of limited use owing to the advanced stage of disease at time of BMAT. NK cell and CD25 assays may, however, assist in raising the diagnostic pickup rate of HLH, especially if patients do not meet the criteria for the remaining parameters.

Additional investigations were performed on all patients in the search for a trigger for suspected sepsis/SIRS before the diagnosis of HLH was made. These investigations varied between treating physicians and were probably focused according to individual clinical symptoms and comorbidities. The search for infective triggers involved testing for herpesviruses, tuberculosis (TB), aerobic micro-organisms and fungi. Members of the herpesvirus family coincidentally are the most frequent triggers of viral-associated HLH. Epstein-Barr virus (EBV), the reactivation of which has been implicated and 3.07×10⁹/µl at the time of fungal culture sampling, while case 4 had a CD4⁺ count of 586 cells/µl with a VL of <20 copies/ml, but he had previously been treated for KS, according to clinical information provided. HHV-8 has been shown to act as an HLH trigger in HIV-positive cases in a limited case series. However, the median CD4⁺ counts of the patients was significantly lower (200 cells/µl). Case 1 was HIV-negative on ELISA and p24 antigen-negative at admission. Recent repeat HIV testing revealed that he is HIV-positive with a CD4⁺ count of 371 cells/µl and a VL of 104 copies/ml. Hence, HLH may have been triggered by either seroconversion or malignancy.

The treating physicians were contacted immediately after the diagnosis of HLH was made and were advised to urgently seek treatment advice from a clinical haematologist. Unfortunately, all patients were at advanced stages of disease at the time of BMAT and cases 2 - 4 succumbed to their illness shortly thereafter. Cases 1 and 5 received appropriate treatment after diagnosis and were alive at time of writing.

The normal immune response to antigenic stimuli (viz. viruses, bacteria or malignancies) involves the clonal expansion of specific T-lymphocytes along with the generation of cytokines to recruit macrophages to the site of concern. A defect in CTL and NK cell-directed apoptosis (via perforin and granzyme) of these activated clonal lymphocytes once the stimulus has been removed forms the common pathway of familial and acquired HLH. Defective apoptosis results in increasing production of pro-inflammatory cytokines, resulting in a catastrophic cascade of events, terminating in multiorgan failure and death. HLH is considered to be rare, and much of what is known in the literature is found in paediatric journals concerned with the familial form. As such, acquired HLH remains a largely unknown entity in adult medicine and its diagnosis is frequently missed in ICU settings.

This limited case series suggests that adult acquired HLH may be a common pathology in acute care settings, where it may masquerade as sepsis or SIRS. Unless it is diagnosed early and appropriate treatment is instituted, the mortality rate is high. Much of the information regarding chemo-/immunotherapy has been gleaned from the HLH-94 protocol where children under 16 years of age were administered etoposide, dexamethasone and cyclosporine (while awaiting a stem cell transplant if diagnosed with familial HLH or relapsing/non-responding secondary HLH). A 5-year survival rate of 54% (standard deviation ±6) was achieved with the HLH-94 trial – a remarkable improvement when previously the mortality rate approached 100%. Given the complex nature of the condition, treatment requires the expertise of a clinical haematologist or medical oncologist well versed in the complexity and challenges of HLH if cure is to be achieved. The concept of using chemotherapy to treat a non-neoplastic condition may seem drastic to medical personnel not familiar with the disease, but its use is essential to induce apoptosis in the defective NK cells and CTLs and quell the cytokine storm.

Establishing a diagnosis of HLH may be difficult in the early stages of disease, as most diagnostic parameters (apart from the gene-specific assays) are in themselves fairly nonspecific. In the
case of oncology patients or those with HIV, the list of differential pathologies is considerable. Only when seen together does a picture of immune hyperstimulation and resulting multiorgan failure emerge.

Possible methods to increase the diagnostic pick-up rate of acquired HLH include auto-flagging of haematological results that exceed upper limits for certain diagnostic criteria (e.g. raised ferritin in the presence of low fibrinogen levels). Alternatively, a standard algorithm for work-up of cases of suspected sepsis with unresolved pyrexia and organomagaly could be instituted in acute care settings. Though not part of the HLH diagnostic criteria, the absence of liver dysfunction in a suspected HLH patient should prompt treating physicians to consider alternative diagnoses.

It is important to note that in this case series, patients received a diagnosis of HLH only after BMATs were performed to further investigate cytopenias or possible septic foci. It is not known how many HLH cases may have gone undiagnosed in patients who revealed no clinical evidence of immune hyperstimulation and resulting multiorgan failure emerge. Only when seen together does a picture of immune hyperstimulation and resulting multiorgan failure emerge.

It is hoped that this case series raises awareness of adult acquired HLH in intensivists, oncologists, physicians and infectious disease experts.
