CYTOMEGALOVIRUS ASSOCIATED HAEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN THE IMMUNOCOMPETENT ADULT MANAGED ACCORDING TO HLH-2004 DIAGNOSTIC USING CLINICAL AND SEROLOGICAL MEANS ONLY

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Haemophagocytic lymphohistiocytosis (HLH) describes a rare, poorly recognised and under-diagnosed immunopathological syndrome whereby there is a highly stimulated yet ineffective multisystem inflammatory response [1]. I present the first case in English literature of Cytomegalovirus (CMV) associated HLH diagnosed by clinical and serological means, and the fourth case of CMV associated haemophagocytic lymphohistiocytosis in an immunocompetent adult, according to HLH-2004 diagnostic guidelines. I include a literature review of CMV associated HLH in adults and raise awareness of checking serum ferritin in patients who present with a sepsis like syndrome. Additionally, this article discusses the merits of HLH-2004 diagnostic work-up without bone marrow biopsy, using clinical and serological means only. I support the reclassification of HLH alongside the other hyperinflammatory syndromes of SIRS, sepsis, septic shock, and MODS to improve understanding and recognition.

Keywords: haemophagocytic lymphohistiocytosis, HLH, haemophagocytosis, inflammation, infection associated haemophagocytic lymphohistiocytosis, IAHS, cytomegalovirus, CMV

Introduction

Haemophagocytic lymphohistiocytosis (HLH) describes a rare immunopathological syndrome whereby there is a highly stimulated yet ineffective multisystem inflammatory response. HLH results in multiorgan dysfunction and haemophagocytosis with the reticulendothelial system characterised by pancytopenia and organomegaly. Incidence has been estimated at 1.2 cases per million individuals per year [1]. Infection-associated haemophagocytic syndrome (IAHS) is a subtype of secondary HLH. Most cases of IAHS are due to viral infections, the majority occurring in the immunosuppressed or children, mostly frequent occurring secondary to Epstein–Barr virus. Along with other types of HLH, IAHS is poorly recognised with high mortality estimates. A recent study demonstrates a mortality of 47% with infection associated HLH [2].

HLH is often fatal if not promptly treated with immunosuppressives. The syndrome is often poorly recognised as the condition mimics a sepsis like syndrome; therefore, it is difficult to accurately estimate incidence [3].

In the last decade, there has been a new diagnostic protocol for HLH to fit the new genetic/molecular immunological advancements. Early, effective therapy using this new HLH-2004 guideline has reduced HLH mortality from 95% to 30–35% (see *Table 1*). Since implementation of HLH-2004 guidelines, it is not mandatory to demonstrate histological evidence of HLH to make the diagnosis of HLH; however, bone marrow biopsy is still regarded as an integral investigation [1]. HLH is associated with extraordinarily high ferritin values, shared with lymphoma and adult onset still's disease only. Ferritin levels greater than 10,000 were associated with 90% sensitivity and 90% specificity for HLH in paediatric patients [4].

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Table 1. HLH-2004 diagnostic criteria [1]

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

1. A molecular diagnosis consistent with HLH
2. Diagnostic criteria for HLH are fulfilled (five out of the eight criteria below)*:
  - Fever
  - Splenomegaly
  - Cytopenias (affecting ≥ 2 or 3 lineages in the peripheral blood):
  - Haemoglobin < 90 g/l (in infants < 4 weeks: haemoglobin < 100 g/l)
  - Platelets $< 100 \times 10^9/l$
  - Neutrophils $< 1.0 \times 10^9/l$
  - Hypertriglyceridaemia and/or hypofibrinogenaemia:
  - Fasting triglycerides ≥ 3.0 mmol/l (i.e. ≥ 265 mg/dl)
  - Fibrinogen ≤ 1.5 g/l
  - Haemophagocytosis in bone marrow or spleen or lymph nodes
  - Low or absent NK-cell activity (according to local laboratory reference)
  - Ferritin ≥ 500 $\mu\text{g/l}$
  - Soluble CD25 (i.e. soluble IL-2 receptor) $\geq 2,400$ U/ml**

*Supportive criteria include neurological symptoms and cerebrospinal fluid pleocytosis, conjugated hyperbilirubinaemia, and transaminitis, hypoalbuminaemia and hyponatraemia, elevated D-dimers, and lactate dehydrogenase. The absence of haemophagocytosis in the bone marrow biopsy does not exclude a diagnosis of HLH

**New data show normal variation by age. Level should be compared with age-related norms

Cytomegalovirus (CMV) is an established precipitant of HLH in immunosuppressed or children, however is incredibly rare within the adult immunocompetent group [3]. I describe case of CMV associated HLH in an immunocompetent adult female, essentially presenting as fever, hepatosplenomegaly, and recurrent pancytopenia. This is the fourth case described in English literature according to HLH-2004 diagnostic guidelines and the first case in English literature to diagnose HLH without bone marrow aspiration, and the patient is treated successfully albeit with prolonged recovery. I then perform a literature review of CMV associated HLH within the immunocompetent adult.

Case report

First admission

A 48-year-old woman presented with gradual onset lethargy, dyspnoea, and right upper quadrant abdominal pain preceded by arthralgia over the past week since return from holiday a week ago. She had been prescribed amoxicillin in the community during the preceding week. She had visited the East coast of the United States with her husband. They spent a week on a cruise ship on their return leg of the journey. She had been subject to fly/mosquito bites.

Her past medical history included Irritable Bowel Syndrome and benign leiomyoma in 2006, she required no regular medication, and she had no known drug allergies. She was independent, lived with her husband; her grown up daughter lived away from home. She was a non-drinker and non-smoker. Upon initial observations, she

was pyrexial at 40 degrees, heart rate (HR) 101 beats per minute (bpm), blood pressure (BP) 133/72 mmHg respiratory rate (RR) 18 breaths per minute (bm), and oxygen saturations (SpO₂) 96% on room air (RA). Upon systemic examination, we noted jaundice and a tender epigastrium. Initial investigations revealed bicytopenia and cholestatic transaminitis with a positive urine dipstick revealing protein and blood (see *Table 2* for the clinical and serological trends throughout first admission). Abdominal ultrasound revealed a slightly thickened gallbladder, 4 mm in diameter, consistent with cholecystitis. Chest X-ray demonstrated a right pleural effusion. Computer tomography (CT) abdomen demonstrated colitis in the ascending colon, a slightly prominent terminal ileum along with bilateral pleural effusions with underlying consolidation. Surgeons responsible for her care diagnosed her with biliary sepsis complicating cholecystitis, and she was treated with intravenous (IV) co-amoxiclav and metronidazole (see *Table 3* for antibiotic regimens throughout first admission). Serum ferritin day 4 post admission was noted to be $> 40,000$ $\mu\text{g/l}$. An exhaustive serological screen for infections including blood cultures, mycobacterial culture, hepatitis A immunoglobulin M (IgM) antibody, hepatitis B surface antigen immunoglobulin G (IgG) antibody, hepatitis C IgG antibody, chlamydia IgG antibody, toxoplasma IgG antibody, parvovirus B19 IgM antibody, leptospira IgM antibody, and borrelia burgdorferi IgG/IgM antibody all returned negative. Complement fixation testing on adenovirus, coxiella burnetti, psittacosis, and herpes simplex virus all showed titres not diagnostically elevated as a single sample. Epstein–Barr nuclear antigen IgG antibody returned positive, indicating previous exposure.

Table 2. Clinical and serological parameters throughout first admission

Day	1	2	4	9	10	12	13	15	18	19	20	30	31	41	47
Fever	Y			Y	Y			Y				Y			
Hepatomegaly					Y						Y				
Splenomegaly					Y	Y									
Haemoglobin (g/dl)	12.7			8.4		6.7	8.4		10.2	9.1	6.9	4.6	10.3	12.5	13.4
White cell count ($\times 10^9/l$)	7.7			6.2		6.2	5.6		3	3.2	2.4	6.7		2.0	3.0
Neutrophils ($\times 10^9/l$)/PV (mPa.s)	4.95			4.24		4.4	3.5		2.0	2.69	1.82	6.34		1.46/1.57	2.31
Platelets ($\times 10^9/l$)	61			62		44	52		49	31	28	44		135	129
C-reactive protein (mg/l)			182	89		69	121		39	59		20		1.2	13.0
Sodium (mmol/l)	129			129		130	128		134	134	138	134		136	135
Bilirubin ($\mu\text{mol/l}$)	53			80		91	120		126	143	86	87		36	37
Alkaline phosphatase (IU/l)	291			693		505	692		620	582	350	202		419	328
Alanine aminotransferase (IU/l)	142			86		62	62		106	116	66	132		153	162
Albumin (g/l)	25			13		17	14		17	18	15	16		34	36
Prothrombin time (s)				15.7		15.3	14.2			13.1	11.7	21.4		10.9	10.8
Non-fasting triglyceride (mmol/l)	3.9														
Fibrinogen (g/l)		1.4										2.0			
Ferritin ($\mu\text{g/l}$)			>40,000						>40,000						28,092
CMV PCR copies ($\times 10^4/l$)						19,000			<2,000						
Blood transfusion (units)						2	1			3	2	4			
Haptoglobins (g/l)/LDH (U/l)											0.28/1,681				
Reticulocytes ($\times 10^9/l$)											1.7				

Table 3. Antibiotic regimen throughout first admission

Day post admission	1	2	3	4	5	6	7	8	9	0	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	
Co-am	*	*	*	*	*	*	*																																									
Met	*	*	*	*	*	*	*																																									
Clarith								*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Taz								*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Doxy												*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Valgan																				*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Gan																			*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Iv Co-T																			*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
PO Co-T																				*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Mero																																																
Amox																																																

Key: Intravenous co-amoxiclav (Co-am); Intravenous metronidazole (Met); Clarithromycin (Clarith); Intravenous tazocin (Taz); Oral doxycycline (Doxy); Oral valgancyclovir (Valgan); Intravenous gancyclovir (Gan); Intravenous co-trimoxazole (IV Co-T); Oral co-trimoxazole (PO Co-T); Intravenous meropenem (Mero); Intravenous amoxicillin (Amox)

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Table 4. Steroid regimen throughout first admission

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																		
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Key: A = PO Prednisolone 20 mg od; B = PO Prednisolone 30 mg od; C = PO Prednisolone 40 mg od; D = PO Prednisolone 40 mg od; E = IV Hydrocortisone 100 mg TDS; F = IV Dexamethasone 6.6 mg bd

Urine and stool samples returned negative. Auto-immune screens returned negative. Despite a seven-day regimen of broad spectrum antibiotics, she remained pyrexial. She was noted to have crepitations on her right lung base with SpO₂ 97% on 2 l O₂ on day 8 post admission. Repeat chest X-ray demonstrated a right pleural effusion, and on the following day, she acutely desaturated immediately after CT pulmonary angiography (CTPA) to SpO₂ 60% O₂ with an associated arterial blood gas was in keeping with type 1 respiratory failure. Her bloods showed pancytopenic picture. She required Intensive Care Unit (ICU) monitoring, non-invasive ventilation (NIV) and insertion of a right sided chest drain. CTPA showed bilateral pleural effusion with atelectasis. She was suitably fluid resuscitated with colloid and started IV clarithromycin 500 mg twice daily for 9 days and IV tazocin 4.5 g thrice daily for 6 days. Pleural fluid grew no organisms. She slowly improved, however remained persistently pyrexial with associated hepatosplenomegaly. She was transferred from ICU to the ward for less than 12 h but then desaturated in the same manner as previously described and so returned needing ICU monitoring and further NIV.

Further investigations for other infectious agents, including urine antigen testing for legionella, viral swab polymerase chain reaction (PCR) for influenza A, B, respiratory syncytial virus, metapneumovirus, parainfluenzavirus types 1–4, rhinovirus and serological testing for legionella, malaria, glandular fever, flaviviruses, alphaviruses, and rickettsial infections had all returned negative. Mycoplasma IgM antibody testing returned positive but at a level indicating low reactivity; thus, we attributed this result as a false positive due to cross reactivity of other antibodies. Despite this, she was started on a seven-day course of oral doxycycline 100 mg bd.

She became progressively haemodynamically compromised by day 12, necessitating 2 units of blood. The same day, we obtained PCR CMV DNA copies of 1.9×10^4 /l. The following day, CMV IgG/M antibody returned positive with an associated CMV low avidity levels at 0.01 avidity index (AI), thus indicating primary CMV infection. After infectious disease consult, a tentative diagnosis of CMV associated haemophagocytosis was established as the patient met five out of the eight criteria shown in table: fever, hepatomegaly, cytopenia of two cell lines, hypofibrinoginaemia, and ferritin >500 µg/l. She was started on prednisolone 40 mg daily (see Table 3 for entire steroid regimen throughout first admission), valganciclovir 900 mg twice daily, and required several further units of blood. She significantly improved; however, she intermittently spiked a temperature, and chest-X ray on day 15 demonstrated persistent pleural effusions.

She was transferred on day 17 to the infectious diseases ward, stable. Her prednisolone dose was halved to 20 mg daily. The subsequent day she again became progressively hypoxic, with SpO₂ 84% on RA, decreased air entry both lungs and bronchial breathing noted left mid zone and arterial blood gas findings consistent with a type 1 respiratory failure. Chest X-ray demonstrated bilateral pulmonary

infiltrates and she re-started IV tazocin for 2 days. Despite oxygen therapy, she remained hypoxic and required NIV.

She further deteriorated and ultimately returned to ICU on day 20 for intubation and ventilation after a stat dose of IV clarithromycin administered. She received IV Hydrocortisone 100 mg TDS, and she was switched from IV tazocin to IV co-trimoxazole 1.8 g twice daily for 7 days followed by oral co-trimoxazole 960 mg twice daily for 4 days. Valganciclovir was switched to IV ganciclovir 300 mg twice daily for 9 days as it is more suitably absorbed in an inflamed bowel. She again became progressively pancytopenic. Lymphocyte subsets revealed a profoundly T cell lymphopenic picture with T cell 0.232×10^9 /l, NK cell 0.013×10^9 /l, and T helper 0.072×10^9 /l, and direct anti-globulin (DAT) test was positive. She was duly transfused 3 units of blood. She underwent bronchoscopy for which AFB's, Human Immunodeficiency testing, and *Pneumocystis carinii* testing all returned negative. Cytology showed that cytology specimen comprises lymphocytes and alveolar macrophages, and degenerates bronchial epithelial cells. Bone marrow aspiration showed reactive toxic bone marrow changes with no significant haemophagocytosis.

She steadily improved, requiring less additional oxygen. Examination of serial chest X-rays demonstrated bilateral patchy air space opacification and moderate sized bilateral pleural effusions. On examination hepatomegaly was noted. She had a worsening pancytopenia, and so she was transfused a further 2 units of blood. Blood film on that day demonstrated thrombocytopenia only. She was extubated the following day, requiring only 3 l of oxygen to maintain adequate saturations. On day 23, a sputum sample returned showing heavy growth of Coliform bacilli with mixed oral flora.

The next day, she improved, saturating at 97% O₂ room air and returned to the ward. Repeat ultrasound (USS) showed normal liver and spleen. On day 27, IV ganciclovir was changed back to valganciclovir and IV dexamethasone was replaced with oral prednisolone 40 mg twice daily. Unfortunately, she became increasingly pancytopenic; on day 29, we halved her steroid regimen to 40 mg once daily, and by day 30, she became less responsive to commands with associated pyrexia and haemodynamic compromise. Chest X-ray was clear. We considered bacterial meningitis and gave IV amoxicillin to cover for bacterial meningitis until CT Head was found to be normal. She returned to IV ganciclovir instead of oral valganciclovir for a total course of 4 days. In light of a recent sputum sample which grew *Enterobacter cloacae* resistant to co-amoxiclav and tazocin, we instituted a seven-day course of meropenem. She was transfused 4 units of blood and was re-admitted to ICU for closer monitoring and central line insertion. She improved and returned to the ward on day 32. Of note, we did not alter her steroid regimen and continued on prednisolone 40 mg once daily for 11 days in total.

She continued to improve; on day 40, we decreased her prednisolone to 30 mg once daily for 5 days. She had persistently deranged liver function tests; on day 41, she had

Table 5. Clinical and serological parameters throughout second admission

Day	1	2	8	12	14	30	38	6 months post follow-up
Fever	Y		Y					N
Hepatomegaly			Y			Y		N
Splenomegaly	Y					Y		N
Haemoglobin (g/dl)	13.1			6.1		11.2	10.6	14.0
White cell count ($\times 10^9/l$)	3.35			5.2		2.8	3.6	4.0
Neutrophils ($\times 10^9/l$)/PV (mPa-s)	5.1			4.09		1.56	2.29	1.82
Platelets ($\times 10^9/l$)	246			96		271	292	164
C-reactive protein (mg/l)	9.4							0.5
Sodium (mmol/l)	139			132		138	136	137
Bilirubin ($\mu\text{mol/l}$)	30			33		10	15	4.1
Alkaline phosphatase (IU/l)	315			292		95	99	42
Alanine aminotransferase (IU/l)	228			166		95	104	42
Albumin (g/l)	36			25		33	34	41
Prothrombin time (s)		11.6		11.9				
Non-fasting triglyceride (mmol/l)								
Fibrinogen (g/l)								
Ferritin ($\mu\text{g/l}$)		>40,000						
CMV PCR copies ($\times 10^4/l$)	Nil				< 2×10^3			
Blood transfusion (units)				2				
Haptoglobins (g/l)/LDH (U/l)					0.06			

an associated alkaline phosphatase (ALP) of 419 IU/l and alanine transaminase (ALT) of 153 IU/l. On day 45, we reduced her steroid regimen to prednisolone 20 mg daily, and she was discharged on day 47 on a reducing course of prednisolone.

Second admission

A week thereafter she was directly re-admitted to the ward with fever, dry cough, right sided chest pain and malaise. Of importance she had reduced her prednisolone to 15 mg daily. On examination she was pyrexial with a temperature of 39.5 °C, BP 121/82 mmHg, HR 145 bpm, RR 24 bpm and SpO₂ 97% on RA. She presented with cervical lymphadenopathy and inflamed tonsils. Bloods revealed leucopenia and transaminitis (see *Table 5* for clinical and serological parameters during second admission). She was treated as a respiratory tract infection with IV meropenem for 8 days. Repeat CMV IgG/IgM antibody returned positive with associated IgG avidity of 0.11 AI, yet CMV PCR returned negative; her ferritin was noted to be >40,000 $\mu\text{g/l}$ once again. Repeat lymphocyte subsets revealed a lymphopenic picture but improved relative to the last admission with T cells $0.866 \times 10^9/l$, NK cells $0.036 \times 10^9/l$ and T helper cells $0.220 \times 10^9/l$.

Repeat serological infection screen returned negative, including Mycoplasma IgM antibody. On day 12 she had

become increasingly pancytopenic. She was treated with 2 units of blood and doxycycline 100 mg twice daily was started for 23 days in total. She spiked a temperature a few hours following transfusion of first unit of blood; she was transfused a further unit thereafter. IV Meropenem was restarted in light of the temperature spike for a total of 20 days. By day 30 one could still detect hepatosplenomegaly on clinical examination despite no pyrexia and improved blood results. She remained on prednisolone 15 mg once daily throughout her second admission. Thereafter she slowly recovered. She was discharged day 40 post admission with persistent hepatosplenomegaly, normal full blood count but persisting transaminitis with a 6-week reducing prednisolone course. Outpatient follow-up has been characterised by a slow recovery. At 6-week follow up, her hepatosplenomegaly resolved, radiologically confirmed by USS abdomen; however, a mild transaminitis persisted, with complete resolution of liver function tests after 6 months (see *Table 4*).

Discussion

HLH is a syndrome of high morbidity and mortality and under-diagnosed [1], owing to a confusing presentation akin to a sepsis-like syndrome, a historical reliance of bone marrow biopsy in order to make the diagnoses and a lack of awareness driven by the scientific community's

segregation of the syndrome from other hyper-inflammatory states.

HLH is divided into primary and secondary forms. Primary HLH, also known as familial, is an inherited condition of impaired lymphocyte cytotoxicity (Natural Killer (NK) cell/CD8⁺ T cell defects) that can also be initial presentation of congenital immunopathologies such as X-linked lymphoproliferative syndrome. Most cases occur in childhood. Secondary HLH arises due to an acquired precipitant, namely infection, haematological malignancy, metabolic or auto-immune disease. Both types have identical phenotypes characterised by inept NK cell / CD 8⁺ T cell responses that lead to macrophage hyper activation, proliferation and infiltration into various organs within a hypercytokinaemic environment [1]. In 1939, the first case of HLH was described as histiocytic medullary reticulosis, in which the patient presented with fever, pancytopenia with a histological finding of haemophagocytosis on a post-mortem examination of bone tissue [5]. Familial HLH was originally described in 1952 as familial haemophagocytic reticulosis [6]. Originally regarded as a fulminant condition caused by malignant histiocytes showing some degree of haemophagocytosis. Later in the 1970s authors such as Warnke et al. described cases in which the patients did not die and demonstrated bone marrow smears in which proliferating histiocytes lacked malignant features, instead exhibiting prominent phagocytosis. Risdall et al. (1979) were the first to describe reactive haemophagocytosis through a case series of 19 reports of which ten immunosuppressed cases of CMV associated HLH (in renal transplant recipients who had undergone splenectomy) were noted [8].

Since the first description of perforin gene mutations in 1999 there has been great progress seen in molecular

biological techniques, as shown by the discovery of the genetic and immunological molecules responsible for the HLH phenotype. All known genetic defects associated with the HLH phenotype encode proteins are involved in the cytolytic secretory pathway. However 20–50% of involved genes are yet unknown and the pathogenesis of the condition remains to be elucidated; theories include either a defective termination of the immune response or failure to remove antigen [1].

Over the last decade, the division between primary and secondary HLH has become increasingly blurred. Research has shown cases of “secondary” HLH in patients with underlying inherited perforin gene defects. Indeed, some reports have shown genetic forms of HLH could be triggered by infection in adulthood [9]. CMV associated HLH is an established phenomena among the Immunosuppressed or Children. Cases occurring in immunocompetent adults are few and far between. An extensive search through PUBMED and references from relevant articles yielded two case reports within the English language which meet HLH-2004 diagnostic criteria and a retrospective review of 99 patients in which there was one case [2, 9, 10]. See *Table 3* for characteristics of each case. I found a Japanese case report, which is unfortunately untranslatable [11], as well as two potential articles in untranslatable languages [12, 13]. I excluded cases with concomitant co-infections. Yu-Tzu Tseng et al. [2] identified one case of CMV associated HLH in an adult in a retrospective review of 99 cases of bone marrow positive HLH cases admitted to National Taiwan University Hospital over an eight-year period (2000–2007). Unfortunately individual characteristics of the cases were unavailable, though inclusion of subjects was dependent on HLH documented on bone marrow biopsy. Hot et al. [9] treat a 32-year-old woman,

Table 6. Individual characteristics of previously reported Cytomegalovirus associated HLH in the immunocompetent adult [2, 9, 10]

Reference	Age/ gender	Medical history	Site of involve- ment	Diagnostic criteria for CMV	Diagnostic criteria for HLH	Treatment	Outcome
Tsuda and Shirono 1996 [10]	21/male	Nil	Lymphadenop- athy (cervical)	CMV IgG and CMV IgM positive	Fever Splenomegaly Bicytopenia Ferritin 1314 BMB positive	Ciclosporin and G-CSF	Discharged day 14, recovered
Hot et al. 2008 [9]	32/ Female	Nil	Skin rash Haematological Transaminitis Splenomegaly	CMV visualized within leucocytes. CMV IgM + Low avidity CMV IgG CMV PCR 41,000 copies/ml	Fever, splenomegaly, Bicytopenias, Ferritin 88,300 Hyponatraemia trans- aminitis BMB positive	IV immuno- globulin for 4 days	Discharged day 15, recovered
Yu-Tzu Tseng et al. 2011 [2]	Not de- scribed	Not de- scribed	Not described	Generic criteria*	In accordance with HLH-2004 criteria BMB+, no other de- tails available	Not de- scribed	Not described

*Abnormal measurements of antibody titer, which included elevation of serum IgG level to more than fourfold in sequential tests, presence of serum IgM, and high titer of IgG ($>1:2,560$), high titer of viral load ($>10^4$ copies/ μ l) or positive results of polymerase chain reaction of cerebrospinal fluid with encephalitis

previously fit and healthy with IV immunoglobulin only. Tsuda and Shirono [10] describe a 21 yr. old previously fit and healthy man. All cases with available information presented with fever and demonstrated haemophagocytosis within their bone marrow specimens. They were investigated and treated in a variety of ways, with a variety of outcomes (see *Table 6*). The team was guided towards the diagnosis of CMV induced HLH once we received positive CMV antibodies on day 13 and noted a serum ferritin of $>40,000 \mu\text{g/l}$ from day 4 of admission. The case demonstrates the diagnostic difficulties involved in HLH. Much of the HLH-2004 criteria can be found in patients with SIRS or sepsis; furthermore many patients with HLH will subsequently develop multiple organ dysfunction syndrome (MODS). Checking serum ferritin aided our diagnosis immeasurably. A review of ferritin in paediatric patients found a cut off of $10,000 \mu\text{g/l}$ to be 90% sensitive and 96% specific for the diagnosis of HLH [6]. HLH is uniquely associated with extraordinarily high levels of the acute phase reactant ferritin. Though the study took place with paediatric patients, this should still be applied in adult medicine. Wijsman et al. cite the use of checking serum ferritin in HLH, regardless of type [14]. Cohort/prospective studies within adult population are awaited. We therefore recommend testing serum ferritin in cases presenting with a severe sepsis like syndrome or those not improving with antibiotics.

This case is the first of its kind to diagnose CMV associated HLH without a bone marrow biopsy clearly demonstrating haemophagocytosis. Historically, HLH was a fulminant syndrome, with haemophagocytosis observed within necropathic bone biopsies. This case, using purely clinical and serological means to diagnose, illustrates the improved pathophysiological understanding, recognition and health outcomes of the condition. Bone marrow biopsy represents an uncomfortable, risky, and expensive test compared with checking serum ferritin, yet is used as a mandatory requirement for several recent cohort studies on HLH [2, 15]. Additionally, bone marrow biopsy has questionable value in making a diagnosis of HLH. Goel et al. [16] performed a case control study on haemophagocytosis seen on bone marrow aspiration in patients fulfilling diagnostic criteria for HLH. They found bone marrow haemophagocytosis to be an unreliable indicator for HLH with a specificity of 60%. Gupta et al. [17] found similar results on their retrospective review, with only 58% of bone marrow specimens demonstrating haemophagocytosis in a sample of 500 HLH confirmed patients. I therefore advocate using bone marrow biopsy only when other supportive criteria are insufficient or if suspecting a haematological malignancy associated HLH. Uniquely, our team made the diagnosis according to HLH-2004 guidelines and initiated steroids prior to bone marrow biopsy. We are the fourth case of CMV-associated HLH meeting HLH-2004 diagnostic criteria, the third in which adhered to the shift in understanding of a condition that up until a decade ago rested on a morphological diagnosis. Scientific progress in molecular diagnostics and immunology

has led authors to view HLH as an extreme manifestation of inflammation [18]. Since 2004, researchers have been promoting the re-evaluation of HLH to an increasingly molecular level. This case intends to add supportive evidence to those authors who suggest inflammation is not a single disease but instead represents a group of conditions that leads to an identical end-stage phenotype. They view HLH as the most extreme clinical manifestation of inflammation that includes (in order of severity of inflammation) systemic inflammatory response syndrome (SIRS), sepsis, septic shock, and MODS [18]. Luckily, in this case, the patient was diagnosed and treated in time, albeit with a slow recovery. However, other cases show there HLH as an under-recognised condition. Raschke and Garcia-Orr [19] describe three unfortunate cases presenting with SIRS/sepsis/septic shock, later found to have secondary HLH, all with fulminant outcomes. They conclude there is an under-recognised association of HLH alongside SIRS/sepsis/septic shock. Reclassification has the potential to improve detection and health care outcomes of HLH, as the syndrome would be more likely to be considered in the differential diagnoses of those presenting with a sepsis-like syndrome.

There is a possibility that the patient experienced a mycobacterial co-infection. The investigations yielded a positive mycoplasma IgM during first admission, which we attributed to a false positive. Repeat IgM on second admission returned negative, but as this was outside the window period for convalescence studies, we deemed the result of no clinical significance.

Distinguishing between primary and secondary HLH without a previously known genetic defect is known to be a significant challenge. Perhaps the patient was predisposed to HLH due to some underlying genetic disorder; however, the late presentation goes against this, as most cases of familial HLH occur in infants [18]. Some reports show that genetic forms could be triggered by infection in adulthood [6]. The patient's lymphocyte subset levels demonstrated global lymphopenia but with particular depression within T cells. Unfortunately, the team failed to test NK cell activity, CD-25, evaluate perforin expression or conduct genetic analysis in order to designate the case into either a primary or secondary HLH. Perhaps the patient had an underlying immunological profile predisposed to react to CMV in a phenotype consistent with HLH. In the future, greater access to these tests will allow for specific genetic therapy such as micro-RNA and it will be important to classify HLH into primary and secondary as treatments will differ; however, for the meantime, it is important to remember that initial management is identical for primary and secondary HLH. This case, as well as the previous cases, all treated outside of accordance with HLH-2004 guidelines recommendations. The Histocyte Society created a therapeutic regimen (HLH-94) in 1994 that consisted of two stages. The first stage is remission induction over 8 weeks of initial treatment with etoposide and dexamethasone. Thereafter, the treatment alters depending upon which type of HLH you are treating and

whether there is resolution of symptoms. For secondary HLH, there is no need to continue further treatment if symptoms have resolved and the primary disease is under control; further treatment consists of daily cyclosporine and alternating weekly pulses of dexamethasone and etoposide, and if the case is refractory, then stem cell transplantation is required [18]. Interestingly, we all used different treatments from each other (see *Table 6*). Hot et al. used only immunoglobulin, with a very successful response. Perhaps CMV associated HLH has a particularly favourable result with immunoglobulin as has been evidenced in Epstein–Barr associated HLH seen in children [18]. Tsuda used cyclosporine A and G-CSF. Both cases had similarly duration of hospital stay of approximately 2 weeks, in stark contrast to our patient who remained in hospital for several months. The patient was treated with variable doses of steroids (see *Table 4*), most likely subtherapeutically, as evidenced by persistent pyrexia (in particular following blood transfusion on second admission), cytopenias (requiring in total 14 units blood), and acute deteriorations upon decreasing prednisolone doses on day 18 and day 29 and pyrexia. Unlike previous cases, the patient was administered anti-viral therapy. Several cases in children or immunocompetent individuals use antivirals [5]. We treated with more than five courses of broad spectrum antibiotics during first admission (see *Table 3*) and treated with two courses of meropenem and a 23-day course of doxycycline for a prolonged period upon second admission. The patient was prescribed an excessive amount of antibiotics due to the diagnostic uncertainty that comes with managing rare disorders such as CMV-associated HLH, in particular when there is no demonstrable haemophagocytosis.

Conclusion

CMV associated HLH in the immunocompetent is under-recognised and very rare. HLH should be considered in those with a sepsis like syndrome presentation. HLH can be diagnosed using clinical and serological means only. Serum ferritin is an easy, quick, and cheap test to use that can be seen as a superior replacement to the more invasive and expensive bone marrow biopsy, as a diagnostic test. Studies regarding serum ferritin sensitivity and specificity in adult onset HLH are awaited. There have been successful outcomes from using immunoglobulin only in CMV associated HLH. Reclassification of HLH alongside other hyperinflammatory states could increase awareness and lead to improved health outcomes.

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