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SHORT REPORT

Case series: Congenital enterovirus infection-associated haemophagocytic lymphohistiocytosis and subsequent neutropaenia

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Congenital enterovirus infection can be associated with a pro-inflammatory state triggering haemophagocytic lymphohistiocytosis (HLH). Enteroviruses are also known to cause transient neutropenia in healthy children. Two infants presented with temperature instability, lethargy, thrombocytopaenia, hepatosplenomegaly and evidence of hyperinflammation in the setting of perinatal maternal rash and household contacts with gastrointestinal symptoms. Whilst HLH was successfully treated in both, protracted neutropenia persisted. Immune dysregulation with enterovirus in the neonatal period can provoke the generation of autoantibodies to hematologic cells giving rise to conditions such as autoimmune neutropenia. Sustained neutropaenia, after resolution of secondary infectious forms of HLH, requires investigation for underlying aetiologies.

KEYWORDS
congenital infections, enterovirus, HLH, neutropaenia

1 | INTRODUCTION

Haemophagocytic lymphohistiocytosis (HLH) is a rare, potentially fatal hyperinflammatory syndrome with multiple potential aetiologies.

Enterovirus infections are common, however, infection in pregnancy has not been widely studied. The majority of enterovirus infections in both children and adults are asymptomatic (90%), however, severe infection can cause: carditis, transaminitis, pneumonitis, meningoencephalitis and death [1, 2]. Neonatal infection can be associated with systemic hyperinflammation. Overall, reported symptomatic perinatal enterovirus cases are likely an underestimate of true incidence [1].

Infectious triggers have also been implicated in the pathogenesis of post-inflammatory cytopaenias and autoimmune neutropaenia (AIN). AIN needs to be considered when unexplained infant neutropaenia persists despite a normal count earlier in the neonatal period.

2 | CASE PRESENTATIONS

We describe two cases of term infants found to have congenital enterovirus infection (CEI) meeting criteria for HLH with persistent neutropaenia after resolution of hyperinflammation. The patients underwent extensive infection screening, as clinically/epidemiologically appropriate, to rule out alternative infectious triggers of HLH (Supplementary A).

List of Abbreviations: AIN, autoimmune neutropaenia of infancy; CEI, congenital enterovirus infection; CSF, cerebrospinal fluid; CT, cycle threshold; GIFT, granulocyte immunofluorescence test; GRA, granule release assay; HLH, haemophagocytic lymphohistiocytosis; IVIG, intravenous immunoglobulin; NPA, nasopharyngeal aspirate; PCR, polymerase chain reaction; XIAP, X-linked inhibitor of apoptosis protein.

TABLE 1 Revised HLH-2004 diagnostic criteria.[a]

HLH-2004 diagnostic criteria	Patient A	Patient B
Fever	X	X
Splenomegaly	✓	X
Cytopaenias (≥2 cell lines)		
Hemoglobin < 100 g/L in neonates	✓	✓
Platelets < $100x10^9$/L	✓	✓
Neutrophils < $1x10^9$/L	X	X
Hyperferritinemia > 500 µg/L	✓	✓
OR		
Hypertriglyceridemia > 3 mmol/L	X	X
Elevated soluble CD25 > 2400 U/mL	✓	✓
Haemophagocytosis (bone marrow or other tissue)	✓	NP
Reduced or absent NK cytotoxicity	NP	NP
Other features:		
Elevated transaminases and/or bilirubin	✓	✓
Elevated LDH	✓	✓
Elevated DDimers	NP	✓
Abnormal CSF	NP	✓

✓Criteria present.
X Criteria assessed but absent.
Abbreviations: CSF, cerebrospinal fluid; LDH, lactate dehydrogenase; NK, natural killer cells; NP, not performed.
[a]Henter JI, Horne A, Aricó M, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2007;48(2):124-131. doi: 10.1002/pbc.21039.

2.1 | Case 1

A 4-day-old boy, born at 37 weeks gestation (weight 2.86 kg) with no risk factors for sepsis presented with lethargy, poor feeding, and reduced urine output. He was the second child of non-consanguineous parents of Caucasian ethnicity. At admission, he was persistently hypothermic and hypotonic, with desaturations. Hepatosplenomegaly was found with a liver edge of 3 cm and a palpable spleen tip. Sepsis was suspected and empiric antibiotics and acyclovir were started. Antenatal history revealed a maternal rash and a sibling with diarrhoea and vomiting around the time of delivery.

Investigations revealed elevated ferritin (63,748ug/L), alanine transaminase (1586 U/L), and lactate dehydrogenase (LDH) (8242 U/L) with low fibrinogen (0.6 g/L), haemoglobin (89 g/L) and platelets (19×10^9 /L). Triglycerides were normal. Granule release assay (GRA), measured by CD107a expression in response to CD3 stimulation was aberrant, though normal with phytohemagglutinin stimulation. Soluble CD25 (sCD25) was elevated (5685 pg/L); X-linked inhibitor of apoptosis protein (XIAP), SAP and perforin protein expression was normal and exome sequencing did not reveal an inborn error of immunity associated with HLH. Bone marrow revealed haemophagocytosis with no evidence of malignancy. (Table 1)

Enterovirus was detected in multiple samples: blood polymerase chain reaction (PCR) (min cycle threshold [CT] 35.12) and nasopharyngeal aspirate (NPA), but negative in the stool. Initial NPA PCR, a picornavirus PCR [Qiastat] detecting both rhinovirus and enterovirus, was positive and confirmed enterovirus with specific PCR primers. Enterovirus serotyping was not possible given low-level viraemia. Lumbar puncture for enterovirus PCR was not performed due to coagulopathy.

The child was treated supportively without the need for immunomodulatory therapy. Antiviral therapy and intravenous immunoglobulin (IVIG) were both considered, however, as spontaneous improvement was noted with high viral CT values, these therapies were not administered.

Despite clinical improvement, protracted neutropaenia ($< 1.5 \times 10^9$/L) was evident at 2 months follow-up (Figure 1). AIN was confirmed with a granulocyte immunofluorescence test ([GIFT] HNA1a-positive antibodies), which measures anti-granulocyte antibodies. CEI was postulated to have resulted in both secondary HLH and subsequent AIN.

2.2 | Case 2

A 6-day-old male, born at 36 weeks gestation (weight 2.4 kg) presented with poor feeding upon which a full septic work-up was performed and empiric antimicrobials commenced. There was no fever. The infant was the second-born child of non-consanguineous parents. No risk factors for sepsis were observed. The infant subsequently deteriorated with apnoeas and hypothermia requiring intubation; inotropic support was required. A detailed history noted that the sibling had been unwell with fever and vomiting the week preceding delivery and was febrile up until the infant's birth.

Cerebrospinal fluid (CSF) demonstrated an elevated cell count (18 cells/µL) and enterovirus PCR was positive. PCR (coxsackie B-type 3) was also positive in NPA, stool and blood (CT 28). CSF and blood PCRs are completed via in-house validated assays with enterovirus-specific primers. Enterovirus serotyping was completed at the Colindale National Reference Laboratory. Supraventricular tachycardia was noted and echo demonstrated left ventricular dilatation but normal function. Coagulopathy was seen with platelets $< 10 \times 10^9$/L, fibrinogen 0.3 g/L, DDimer 19,585 ng/mL, as well as anaemia (Hb 56 g/L), lymphopaenia (1.22×10^9 /L), and transaminitis, but no splenomegaly. Ferritin peaked at 1657ug/L, LDH at 14,036 U/L but c-reactive protein and triglycerides were never significantly elevated. A bone marrow was not performed. sCD25 was elevated (4585pg/L) and GRA was abnormal. Perforin, SAP and XIAP protein expression were normal. (Table 1)

Like in case #1, spontaneous clinical improvement occurred with supportive care alone. Serum enterovirus CT values gradually increased and thus IVIG was not administered. In the convalescent phase, persistent neutropaenia (nadir 0.81×10^9 /L) was equally demonstrated (Figure 1), although eventually recovered without intervention and thus a GIFT was not pursued.

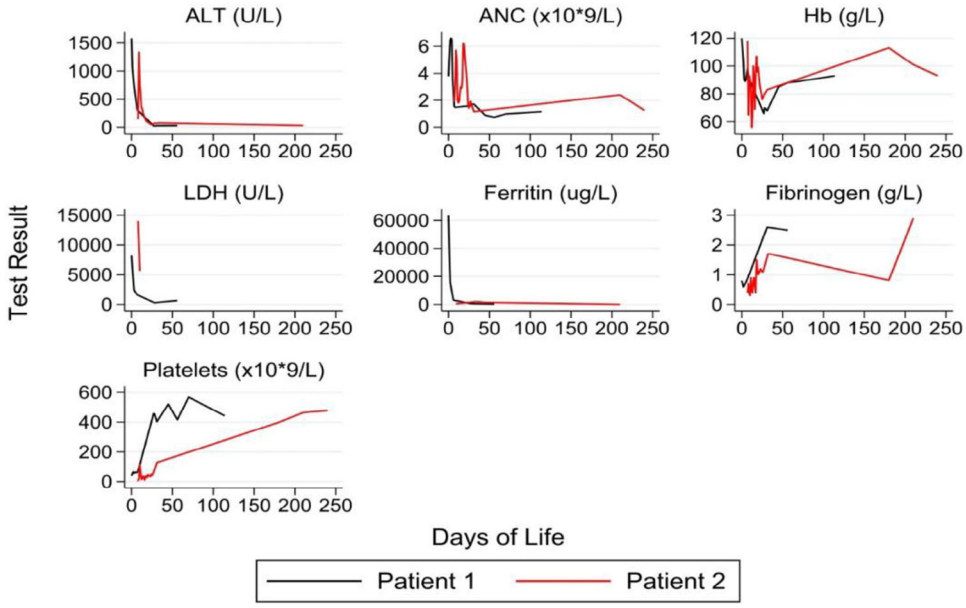

FIGURE 1 Biochemical and haematologic parameters over time. ALT, alanine transferase; ANC, absolute neutrophil count; Hb, haemoglobin; LDH, lactate dehydrogenase.

3 | DISCUSSION

We describe two cases of CEI triggering HLH where, despite the resolution of hyperinflammation, unexplained neutropaenia persisted, one of which was found to have anti-neutrophil antibodies. Our patients demonstrate the unique immune-dysregulation trigger of congenital infections both in the acute hyperinflammatory phase of HLH and in the convalescence phase, potentially contributing to immune-mediated cytopaenias.

HLH is life-threatening, warranting expert supportive care and prompt targeted treatment but often difficult to differentiate from neonatal infections [3]. Both primary and secondary HLH diagnosed in the first four weeks of life carry a poor prognosis with mortality rates up to 60%; time to treatment has prognostic implications and thus requires prompt recognition [4]. Abnormal GRA results in our patients are likely explained by young age but did prompt further immunologic investigations. In addition to genetic analysis for primary causes, investigations for congenital or perinatally acquired infections are imperative. Neonatal enterovirus infection is recognised as one of the most common viral triggers of HLH, along with Herpes simplex virus [5].

Our patients exemplify the importance of a detailed perinatal history. Maternal rash and fever and gastrointestinal symptoms in siblings raised suspicion of CEI. The presentation of CEI masquerading as HLH was similar to those previously described with coagulopathy, hepatosplenomegaly, transaminitis, and hyperferritinemia [6–8]. This can progress to hepatic inflammation potentially requiring liver transplantation, fulminant myopericarditis, and/or coagulopathy. Whereas some infectious triggers of HLH have targeted antimicrobial/antiviral treatments, CEI lacks a definitive treatment and variation in treatment approaches exists. Pleconoril, pocapivir, repurposed medications (i.e. fluoxetine and favipiravir), and IVIG have been given, although spontaneous improvement with supportive care is often observed, as with our patients [9].

Both patients were found to have persistent neutropaenia months after the resolution of HLH, prompting further investigations. A case series of three neonates with confirmed enterovirus sepsis described one patient with HLH but with cytopaenias that resolved at a much faster rate than in case #1 and with no evidence of an autoimmune cause of neutropaenia [7]. Likewise, a separate case report showed equally rapid resolution of abnormal lab values with symptomatic treatment of neonatal enterovirus-triggered HLH [9]. To our knowledge, no cases of persistent neutropaenia after the resolution of initial hyperinflammation have been reported.

AIN is described as one of the most common causes of persistent neutropaenia in infancy [10, 11]. Thought to be commonly triggered by viruses, AIN differs from neonatal autoimmune neutropaenia, caused by transplacental antibodies directed against foetal neutrophils, in that it typically presents outside of the immediate neonatal period [12]. This was demonstrated in both patients, having had normal neutrophil counts at birth, even during the initial HLH episode. Infantile neutropaenia has also been associated with other viruses, particularly the *Herpesviridae* family [13] and hepatitis C [14]. Parvovirus and HIV infection [15, 16] have been implicated in the development of autoantibodies to neutrophils. A case series of two infants with congenital cytomegalovirus and subsequent AIN has also been described [17].

Case#1 was found to have HNA-1a self-antibodies. Autoantibodies against HNA-1a and HNA-1b are most commonly found in AIN, although non-typable autoantibodies can be present [10, 12]. Less frequently, antibodies to adhesion glycoproteins HNA-4a/b, CD35, and FcγIIb are isolated [18, 19]. Enteroviruses are associated with chronic

inflammation with a propensity to induce autoimmunity via induction of pro-inflammatory cytokines of the TH1 and IL10 pathways, altering both cellular and humoral responses [20].

4 | CONCLUSION

Both patients exemplify the importance of an initial broad infection screen in neonates presenting with features of HLH, including a thorough congenital infection screen [21]. Once acute inflammation of HLH has resolved, subsequent immune-mediated pathology (i.e. AIN) must be considered as a cause for persistent cytopaenias.

AUTHOR CONTRIBUTIONS

Justin Penner was the primary author of the manuscript; James E. Burns prepared the tables and figures; Judith Breuer provided clinical virology expertise; Kimberly C. Gilmour provided interpretation of immunological investigations; Alasdair Bamford was the clinical infectious diseases lead; Anupama Rao was the haematology clinical lead; Justin Penner, Judith Breuer, Kimberly C. Gilmour, Alasdair Bamford and Anupama Rao provided direct clinical care to the cases presented. All authors reviewed and approved the final draft manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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DATA AVAILABILITY STATEMENT

All presented data was gathered from patient electronic clinical records. No data has been stored. Additional data and information pertaining to the presented cases are available on written request to the authors.

ETHICS STATEMENT

This retrospective review of patient data did not require ethical approval in accordance with local/national guidelines.

PATIENT CONSENT STATEMENT

Consent was provided by the patients' caregivers for the publication of details of their medical case.

CLINICAL TRIAL REGISTRATION

The authors have confirmed clinical trial registration is not needed for this submission.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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