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CLINICAL LETTER

WILEY

Familial hemophagocytic lymphohistiocytosis type 3 presenting as neonatal cholestasis and splenomegaly

To the Editor,

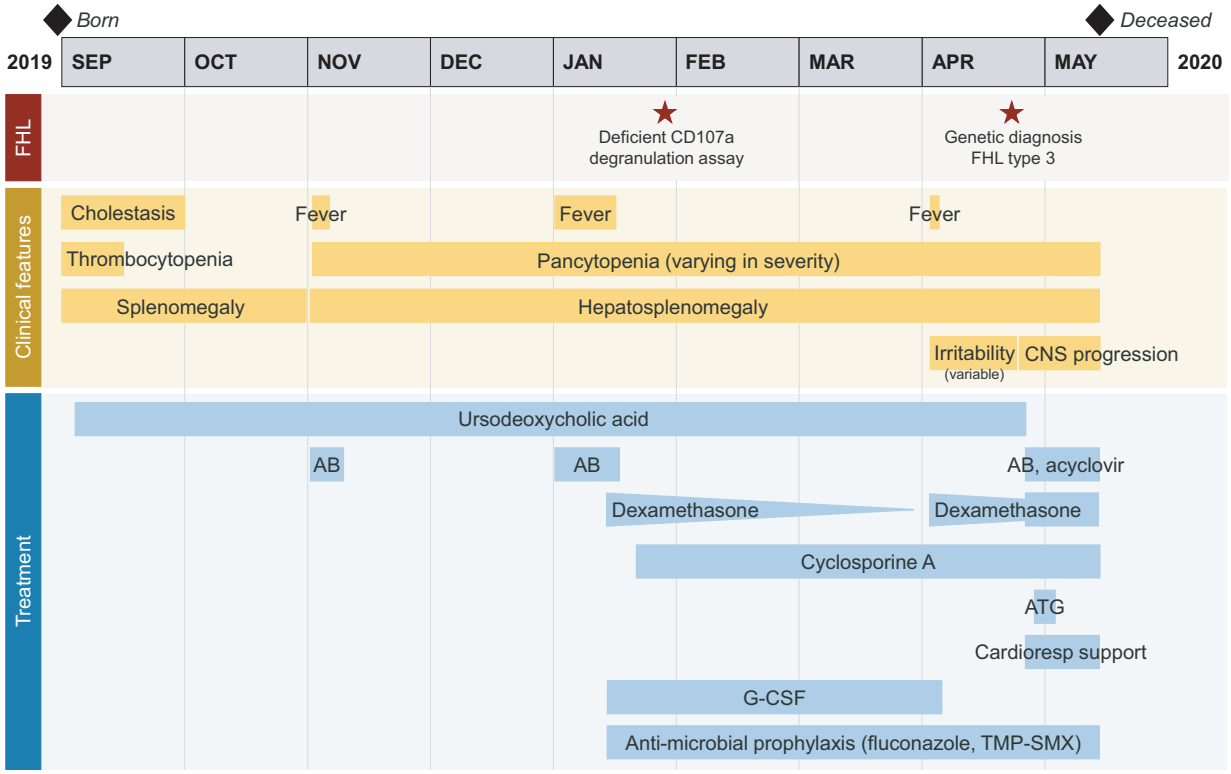
Hemophagocytic lymphohistiocytosis (HLH) is a severe hyperinflammatory condition caused by excessive release of pro-inflammatory cytokines, typically resulting in fever, cytopenia, and hepatosplenomegaly.[1] HLH can be life-threatening due to rapid progression to multi-organ failure. Delay in diagnosis and treatment of HLH has been shown to adversely affect survival.[1] There are several forms of HLH. The term primary HLH is used when there is a genetic predisposition, including familial HLH (FHL) types 1–5 and several pigmentary syndromes and other primary immunodeficiency disorders. Secondary HLH occurs in the context of infections, malignancies, rheumatic diseases, or metabolic disorders, in the absence of a genetic defect.[1] Primary and secondary HLH are clinically indistinguishable, and initial treatment (HLH-1994/2004 protocol) is aimed at damping the cytokine storm. However, timely recognition of the underlying etiology is important for subsequent management choices. In particular, the only curative treatment for primary HLH is allogeneic hematopoietic stem cell transplantation (HSCT).[1] Despite the progress made in this field, the diagnosis of HLH remains challenging and current diagnostic criteria insufficiently cover the diverse clinical manifestations. The latter is especially true for neonates. We here report an infant with an initial presentation of neonatal cholestasis, splenomegaly and transient thrombocytopenia, and a delayed identification of FHL type 3 (FHL3).

The patient is a Caucasian female infant born to non-consanguineous healthy parents at 35 weeks of gestational age. Polyhydramnios was observed during pregnancy. After an uncomplicated birth, she had mild symptoms of respiratory distress during the first 2 days of life. She was referred to our center at day 4 because of cholestasis and marked thrombocytopenia (Figure 1). She received enteral feeding without need for parenteral nutrition. There were no bleeding symptoms, and coagulation screening was normal. Fetal neonatal alloimmune thrombocytopenia was excluded. Abdominal ultrasound demonstrated splenomegaly (bipolar diameter of 7.3 cm), mild hepatomegaly (midclavicular diameter of 6.6 cm), and a normal biliary tract. Further extensive infectious, hormonal, and metabolic workup for neonatal cholestasis was negative. She was treated with ursodeoxycholic acid. Neurologically, the patient showed poor drinking and intermittent dormant behavior. However, MRI of the brain and repeated electroencephalograms were normal, and the neurological symptoms improved overtime. Platelet counts spontaneously normalized by day 15, cholestasis resolved by the end of the first month, but the splenomegaly persisted. She was discharged from the hospital at day 30, at which time she was clinically well. Although no diagnosis was found, a metabolic disorder was suspected based on the combination of neonatal cholestasis, splenomegaly and transient cytopenia, and follow-up at the metabolic clinic was scheduled.

At the age of 2 months, she presented with a first confirmed episode of HLH characterized by high fever, marked hepatosplenomegaly, pancytopenia, hyperferritinemia, and hemophagocytosis in bone marrow (5 of 8 HLH criteria) (Figure 1).[1] Broad-spectrum antibiotics were given for a week, and cultures remained negative. The fever and neutropenia rapidly resolved. Still, hepatosplenomegaly persisted, and white and red blood cell counts remained moderately low. Because of the rapid clinical recovery without immunosuppressive drugs and the manifestations at neonatal age, secondary HLH was suspected. A tentative diagnosis of lysosomal acid lipase deficiency (Wolman disease) was made, a rare lysosomal storage disease known to be associated with secondary HLH.[2]

At 4 months of age, the patient had a second, more severe, HLH episode with neutropenic fever as well as cardiorespiratory and neurological symptoms. Treatment with dexamethasone and cyclosporine A (CsA) was started (Figure 1).[3] Gradual clinical improvement was seen, and no etoposide or intrathecal methotrexate was given. This time, additional investigations were performed and revealed a deficient NK cell CD107a degranulation suggesting primary HLH (Figure 1B).[4] Taken together, the patient fulfilled 6 of 8 criteria during the second HLH episode (no hyperferritinemia, no bone marrow puncture performed). The diagnosis of primary HLH was later confirmed by genetic analysis that showed a homozygous *UNC13D* mutation causing FHL3 or Munc13-4 deficiency.[5] In the meantime, CsA was continued and dexamethasone was slowly tapered over a period of 9 weeks.[3] Search for an allogeneic stem cell donor was initiated. Shortly after stopping dexamethasone, the 7-month-old patient presented again with fever and irritability, and corticosteroids were restarted (Figure 1). In the following weeks, she developed severe central nervous system (CNS) manifestations with progression to refractory seizures and encephalopathy (Figure 2). In addition to high-dose dexamethasone and CsA, antithymocyte globulin (ATG) was administrated.

(A)



(B)

Date	SEP 2	SEP 13	SEP 27	NOV 4	NOV 26	JAN 3	JAN 23	FEB 17	MAR 19	APR 3	MAY 7
Age	4 days	15 days	29 days	2 months	2.5 months	4 months	4.5 months	5 months	6 months	7 months	8 months
Hemoglobuline (g/dL)	15.2	10.0 ↓	9.1 ↓	7.2 ↓	8.3 ↓	7.0 ↓	7.6 ↓	8.2 ↓	7.0 ↓	6.5 ↓	6.7 ↓
Thrombocytes (no./μL)	54,000 ↓	150,000	228,000	33,000 ↓	162,000	23,000 ↓	113,000 ↓	128,000 ↓	214,000	213,000	266,000
White blood cells (no./μL)	8440 ↓	5180 ↓	7190 ↓	2830 ↓	4620 ↓	2260 ↓	8060	13,310	3290 ↓	19,970 ↑	4620 ↓
Neutrophils (no./μL)	954 ↓	1270 ↓	1290 ↓	541 ↓	1502	160 ↓	5102	9510 ↑	1379 ↓	15520	4270
Lymphocytes (no./μL)	4718	n.d.	n.d.	1868 ↓	2241 ↓	1470 ↓	2636 ↓	3090	1727 ↓	3250	240 ↓
Bilirubine, total (mg/dL)	17.2 ↑	4.2 ↑	2.4 ↑	0.6	0.6	0.6	0.4	n.d.	0.4	n.d.	< 0.3
Bilirubine, direct fraction (mg/dL)	9.87 ↑	3.04 ↑	1.52 ↑	0.23	0.22	< 0.1	0.19	n.d.	0.15	n.d.	< 0.1
AST (SGOT) (U/L)	49	80	74 ↑	63 ↑	48	78 ↑	40	41	24	33	38
ALT (SGPT) (U/L)	16	67 ↑	56 ↑	28	35	42	47	45	18	9	28
Ferritine (μg/L)	1053 ↑	n.d.	n.d.	1288 ↑	324 ↑	309 ↑	362 ↑	186 ↑	10	29	144 ↑
Fibrinogen (mg/dL)	156 ↓	n.d.	n.d.	195 ↓	261	149 ↓	106 ↓	172 ↓	140 ↓	288	91 ↓
Triglycerides (mg/dL)	105	n.d.	n.d.	140 ↑	n.d.	170 ↑	453 ↑	n.d.	67	94	420 ↑
Soluble CD25 (pg/mL)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	> 20,000 ↑	n.d.	n.d.	n.d.	n.d.
Perforine expression	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	91.9% on NK cells	n.d.	n.d.	n.d.	n.d.
CD107a degranulation assay *	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.4% on resting NK cells ↓ 15% on activated NK cells (Ki562) ↓	n.d.	n.d.	n.d.	n.d.

FIGURE 1 Overview of the most important HLH-related features. (A) Timeline showing the clinical course and treatment of the patient. Diagnostic milestones are highlighted by a star. (B) Representative laboratory results. It is indicated whether the value is decreased (↓) or elevated (↑) compared with age-based reference values. AB, antibiotics (broad-spectrum); ATG, antithymocyte globulin; CNS, central nervous system; FHL, familial HLH; G-CSF, granulocyte – colony-stimulating factor; HLH, hemophagocytic lymphohistiocytosis; n.d., not determined; TMP-SMX, trimethoprim-sulfamethoxazole. *According to the recommendations of Bryceson et al.[4]

Unfortunately, her neurological condition rapidly deteriorated and she died at 8 months of age.

This report underlines the difficulty of diagnosing primary HLH in neonates and infants.[6] Here, the presence of cholestasis and splenomegaly at neonatal age led to the suspicion of an underlying metabolic disorder, which was strengthened by the rapid improvement of the first HLH episode without corticosteroids. This created a diagnostic blind spot and delayed the identification of FHL3 with fatal consequences.

During the first days of life, the patient fulfilled 3 of 8 diagnostic criteria for HLH: splenomegaly, low platelets and neutrophils, and hyperferritinemia. Fibrinogen was reduced but missed the required threshold. If additional diagnostics for HLH would have been performed at that time, one can assume that sufficient

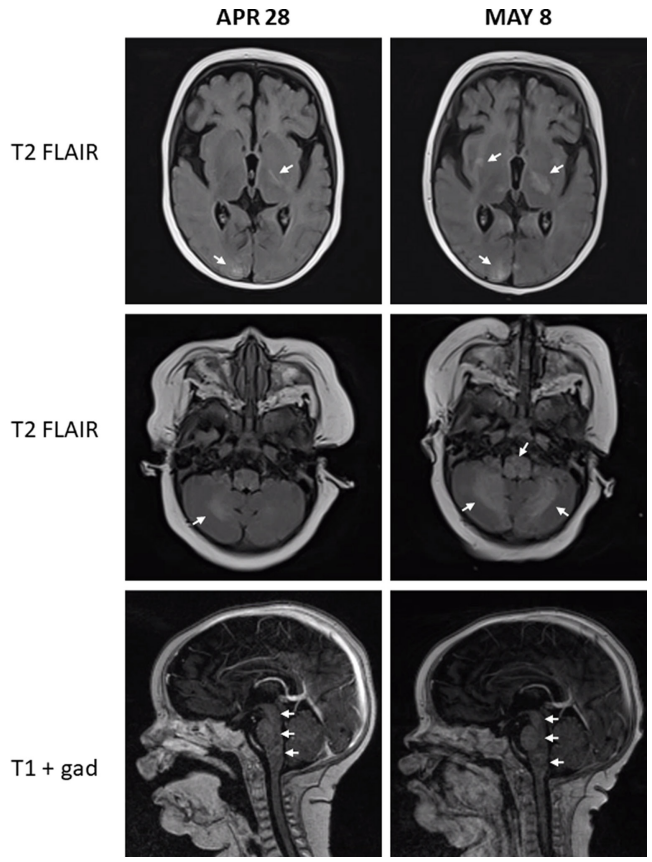


FIGURE 2 CNS-HLH manifestations. Serial MRI images of the brain demonstrate progressive white matter lesions (indicated by arrows) in the cerebral hemispheres, cerebellum and brain stem, compatible with HLH-associated CNS inflammation. CNS, central nervous system; HLH, hemophagocytic lymphohistiocytosis; gad, gadolinium contrast

criteria might have been fulfilled to establish the diagnosis. Still, the absence of fever was atypical. It is known that the clinical presentation of HLH may be atypical or incomplete, that is, not corresponding to the diagnostic criteria proposed by the Histiocyte Society. These criteria were originally intended as inclusion criteria for the HLH-2004 trial, and real-world data on their sensitivity or specificity are missing.[1] Although HLH in neonates is rare, previously published cases demonstrate that hepatic dysfunction and cholestasis are common manifestations in these patients.[7,8] However, neonatal HLH is often misdiagnosed as metabolic liver diseases.[9] Based on our and previous case reports as well as the importance of a timely diagnosis of primary HLH, we recommend that cholestasis should be included as diagnostic criterium for HLH in patients below the age of 1 year. When looking from the other side of the coin, neonatal and infantile cholestasis has an extensive list of differential diagnoses. Still, HLH is not included in the latest NASPGHAN-ESPGHAN consensus recommendations (NASPGHAN, North American Society for Gastroenterology, Hepatology and Nutrition; ESPGHAN, European Society for Gastroenterology, Hepatology and Nutrition).[10] It is only very recently, in the SIGENP position paper of 2022, that HLH is incorporated in an international guideline on neonatal and infantile cholestasis (SIGENP, Italian Society for Pediatric Gastroenterology, Hepatology and Nutrition).[11] Unfortunately, this paper was not yet available at the time we were treating our patient. We strongly believe that the inclusion of (primary) HLH in international guidelines as those of SIGENP will help to increase awareness among physicians and facilitate a more timely diagnosis.

In the here-reported patient, the first documented HLH episode at the age of 2 months was initially assumed to be secondary to a metabolic disorder. However, in the first year of life, primary or genetic forms of HLH are more prevalent than secondary forms, and a complete diagnostic workup should always be performed.[1] In this case, screening for primary HLH was delayed until the second HLH episode at 4 months of age, and the genetic diagnosis of FHL3 was only established at the age of 7 months. Whether an earlier diagnosis of FHL3 would have altered the outcome, will remain uncertain. FHL comprises a group of rare autosomal recessive disorders caused by genetic defects in lymphocyte cytotoxic function. Overall, FHL has a poor prognosis with a 5-year survival of about 50%.[3] FHL3 or Munc13-4 deficiency accounts for about one-third of FHL cases and is caused by biallelic loss-of-function mutations in *UNC13D*.[5] A recent systematic review on FHL3 patients by Amirifar et al. found that the majority of them had fever, cytopenia, and hepatosplenomegaly, like was seen in the here-reported case. More than half of patients suffered from CNS involvement, an unfavorable prognostic factor.[5] Strikingly, the here-reported patient showed progressive central HLH while under immunosuppressive therapy, and additional treatment with ATG had no effect. In the review by Amirifar et al., only 52% of FHL3 patients had a good response to immunochemotherapy.[3,5] It is not mentioned if FHL3 patients with CNS involvement showed a poorer response to treatment than those without.[5] Among those who were able to proceed to HSCT, the posttransplant median survival was estimated to be 14 months.[5] Although the results of this systematic review should be interpreted with caution because of incomplete data in many publications, it is clear that CNS inflammation is a frequent complication in FHL3 patients and that current treatment modalities are unsatisfactory.

In conclusion, we want to raise awareness that neonatal cholestasis can be an early symptom of FHL3, and that pediatricians should keep a high index of suspicion especially when accompanied by persisting cytopenia and splenomegaly. Furthermore, timely diagnosis of primary HLH could be facilitated by including this clinical association in the leading international guidelines on both HLH and neonatal cholestasis, such as the recent SIGENP position paper of 2022.

KEYWORDS
cholestasis, familial hemophagocytic lymphohistiocytosis, hemophagocytic lymphohistiocytosis, Munc13-4, neonatal, UNC13D

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CONFLICT OF INTEREST

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PEER REVIEW

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