

# Hemophagocytic lymphohistiocytosis arising in a child with Langerhans cell histiocytosis

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Langerhans cell histiocytosis (LCH) is characterized by the proliferation of clonal dendritic cells, while hemophagocytic lymphohistiocytosis (HLH) is an extreme inflammatory process sustained by the uncontrolled activation of macrophages. HLH can be primary or secondary, the latter arising in infectious, autoimmune or neoplastic disorders. We hereby present a young girl who developed secondary HLH while being treated for relapsed multisystem LCH under the LCH III Protocol. She fulfilled 5 of 8 HLH-2004 criteria (fever, splenomegaly, pancytopenia, ferritin level  $>500 \mu\text{l}$  and sIL-2R  $>2400 \text{ IU/ml}$ ) and was successfully treated by the HLH-2004 Protocol for secondary HLH. She remains in good health, apart from insipid diabetes she developed as a complication of LCH. Considering that the occurrence of HLH in LCH patients has been reported before, the case history presented here yields additional support for the hypothesis that the pathogenesis of the two histiocytoses – LCH and HLH – may indeed overlap to a considerable extent.

**Key words:** hemophagocytic lymphohistiocytosis, Langerhans cell histiocytosis, child.

Langerhans cell histiocytosis (LCH) is a disease characterized by the proliferation and accumulation of clonal dendritic cells. The etiology is still not completely understood, and there is a long-standing debate as to whether LCH is a neoplastic process or not. Recent findings of recurrent BRAF mutation in LCH patients<sup>1</sup> appear to favor the neoplastic hypothesis. Although rare, LCH is the most common histiocytic disorder. Its clinical features display great heterogeneity and its course is largely unpredictable<sup>2</sup>. LCH may occur as a single organ/system disease with an excellent survival rate<sup>3</sup>, or a disease affecting two or more systems of the body in various combinations and with an unpredictable course. The latter form is designated “multisystem LCH.” The localization and extent of LCH lesions correlate with the clinical course and prognosis. Involvement of the bone marrow, liver and spleen, categorized as ‘risk organs’ in the LCH IV Protocol by the Histiocyte Society, mandates more intensive treatment<sup>4,5</sup>.

In contrast to LCH, hemophagocytic lymphohistiocytosis (HLH) is a disorder

characterized by an inflammatory process caused by the extreme and uncontrolled activation of macrophages<sup>6,7</sup>. The main clinical features are fever, hepatosplenomegaly and cytopenia involving two or more cell lines. Lymphadenopathy, jaundice and neurological symptoms are also fairly common. Hemophagocytosis is defined as the pathological activity of macrophages that engulf and digest erythrocytes, leukocytes, thrombocytes and their precursor cells. Hemophagocytosis is not always present, although it usually appears at some point in the course of the disease. Laboratory findings characteristic of HLH include elevated plasma levels of triglycerides, ferritin, transaminases, bilirubin and lactate dehydrogenase (LDH), together with low fibrinogen levels. More than half of patients also have abnormal cerebrospinal fluid findings (increased proteinorachia with the presence of leukocytes)<sup>8</sup>. Depending on the existence of an underlying condition, HLH can be either primary (genetic) or secondary (acquired). Primary HLH is either familial (FHLH), which may be caused by mutations in perforin, Munc

13-4, syntaxin 11 or STXBP2; or associated with some primary immunodeficiency disorder, such as Chédiak-Higashi syndrome, Griscelli syndrome, Hermanski-Pudlak syndrome or X-linked lymphoproliferative syndrome. In approximately 50-60% of patients affected by genetic HLH, the underlying defect is unknown<sup>7</sup>. Secondary HLH can be encountered in association with a number of different disorders, including infections (most commonly by herpesviruses, such as Epstein-Barr virus, cytomegalovirus, herpes simplex virus and varicella-zoster virus; also by *Leishmania*), as well as autoimmune disorders (where it is often designated “macrophage activation syndrome”) and malignancies<sup>9,10,11</sup>. The involvement of as yet unknown genetic factors in secondary HLH cannot be ruled out.

### Case Report

A female child presented at the age of 2 months with a red papular skin rash in the abdominal, cervical and inguinal regions, as well as on the palms of the hands and soles of the feet. Other physical examination findings were normal. Personal and family histories were unremarkable. Blood counts were in the age-dependent reference ranges. Routine biochemistry tests, e.g., blood glucose, urea, creatinine, sodium, potassium, total protein, albumin, total bilirubin, direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase and gamma-glutamyl transferase (gamma-GT), showed no abnormal values. Chest X-ray examination was unremarkable, as was the abdominal ultrasound. Upon pathohistological examination of the cutaneous areas affected by the rash, the diagnosis of LCH was established. However, the full diagnostic examination required by the Histiocyte Society LCH Protocol was not performed at that time. She was treated by her dermatologist with combined topical glucocorticoid treatment and prednisone 1 mg/kg/day 3 times daily. This treatment soon resulted in clinical improvement. However, nine months later, due to a lower jaw swelling with erythema and tenderness of the overlying skin, the child was referred to the University Children’s Hospital. X-ray imaging of the head and neck revealed an osteolytic lesion in the right mandible. Two additional osteolytic foci were discovered in

the right hemicranium (located parietally) (Fig. 1). A skeletal radiographic survey showed that other bones were apparently free of lesions. Also, an aural discharge on the left side was seen and found to have already been present for several months and refractory to standard treatment. Ultrasonographic examination of the neck measured right cervical lymph nodes at around 12 mm in diameter. WBC count was  $13.9 \times 10^9/L$  with 27% neutrophils, 67% lymphocytes, 4% monocytes and 2% eosinophils. RBC count was  $5.2 \times 10^{12}/L$ , hemoglobin concentration 11.3 g/L, and platelet count  $330 \times 10^9/L$ . Serum iron concentration was 8.1  $\mu\text{mol}/L$ , transferrin 2.8 g/L, and ferritin 12.1 mg/ml. Routine biochemical tests were normal, as were urine osmolality and blood coagulation screening tests. She was attributed to the “multisystem low-risk” LCH group and treated accordingly under the LCH III Protocol (vinblastine 6 mg/m<sup>2</sup> weekly for 6 weeks and prednisone 40 mg/m<sup>2</sup>/day orally for 4 weeks, tapering off over 2 weeks: “initial treatment course 1”). Upon evaluation, disease regression was found according to the criteria of the treatment protocol, and she was given continuation treatment (prednisone 40 mg/m<sup>2</sup>/day in three doses for 5 days every three



Fig. 1. Anteroposterior X-ray image showing osteolytic parietal and mandibular lesions of Langerhans cell histiocytosis (arrows).

weeks and vinblastine 6 mg/m<sup>2</sup> once every three weeks). However, five months after the continuation treatment had begun, polyuria (5990 ml/day) and polydipsia (6950 ml/day) were noted, serum osmolality was 263 mosm/l, urine osmolality 73 mosm/l and diabetes insipidus was diagnosed. Antidiuretic hormone replacement was administered continuously. Continuation treatment with prednisone according to the LCH-III Protocol was extended to 12 months by clinical decision. Regularly performed follow-up examinations showed normal physical and laboratory findings, as well as regression of bone lesions.

Nine months after the completion of therapy, at the age of 2 years and 7 months, swelling of the right mandible appeared once again, accompanied by fever. Biopsy of this lesion was performed, confirming that this was indeed a relapse of LCH. Full evaluation failed to find any other lesions in tissues or organs. The child once again underwent chemotherapy, beginning with "initial treatment course 1" (see above). This treatment swiftly rendered the child nonfebrile. Upon evaluation, she was declared "better" and given continuation treatment, consisting of 6-mercaptopurine (50 mg/m<sup>2</sup>/day) and methotrexate (20 mg/m<sup>2</sup>/week). Follow-up examinations, performed regularly thereafter, have demonstrated a stable regression of bone lesions.

At the age of 3 years and 4 months, while still undergoing maintenance therapy for relapsed LCH (week 13), the patient was hospitalized because of fever. Physical findings were unremarkable. WBC was 3.8 x 10<sup>9</sup>/L (lymphocytes 40.2%, monocytes 7.1%, granulocytes 52.7%). RBC count was 2.9 x 10<sup>12</sup>/L and hemoglobin 99 mg/dl. Platelet count was 151 x 10<sup>9</sup>/L. ESR was 17 mm and CRP 62 mg/dl. Maintenance therapy was discontinued. Blood culture results were negative. In the absence of an obvious focus of infection, ceftriaxone was administered, but after 72 hours of febrility, with CRP reaching 164 mg/L, meropenem was introduced instead. Five days after admission, watery diarrhea and abdominal pain ensued. Stool culture results were negative for bacterial infection. Latex agglutination-based stool tests did not detect rotaviruses or adenoviruses. The patient's diarrhea raised suspicion of possible *C. difficile* infection.

Since a stool test for specific toxins was not available, and the patient's general condition at the time did not permit bowel endoscopy with pathohistological examination of the mucosa, vancomycin was added to the therapy on an empirical basis. This appeared to have been successful: two days later, fever and diarrhea subsided. However, a single, spontaneously resolving episode of generalized tonic-clonic convulsions ensued. Blood glucose level was within normal range and sodium concentration was measured at 120 mmol/l. EEG examination yielded signs of CNS suffering: slow brain activity with superposition of fast rhythm and sharp cortical  $\theta$ -waves, predominantly on the right side. CSF examination findings were normal, and there were no intracranial lesions detectable by CT scan. No further seizures occurred after sodium level correction.

Splenomegaly was then noted and confirmed by ultrasonography. The spleen was enlarged (craniocaudal diameter was 120 mm; the upper limit for her age and height is 90 mm). The liver craniocaudal diameter was at the upper limit (110 mm), and liver structure was homogenous. Several lymph nodes measuring up to 11 mm were noted in the porta hepatis and the mesentery. The child's blood cell counts began to plummet. WBC count fell to 2.3x10<sup>9</sup>/L (neutrophils 25% or 0.58x10<sup>9</sup>/L; lymphocytes 58% or 1.34x10<sup>9</sup>/L; monocytes 17% or 0.39x10<sup>9</sup>/L). RBC count fell to 2.84x10<sup>12</sup>/L, with Hb 88 g/L, while platelet count reached 92x10<sup>9</sup>/L. Concentration of IgA was 0.32, IgG 6.8, IgM 0.62 g/L. Serum ferritin level was 517.2  $\mu$ g/L. Plasma fibrinogen level was measured at 7.04 g/L. Antinuclear antibodies were absent (by indirect immunofluorescence). Complement components C3 and C4 were within normal range. Lymphocyte immunophenotyping showed that absolute numbers of B cells (CD19<sup>+</sup>) and NK cells (CD3<sup>+</sup>CD16<sup>+</sup>/CD56<sup>+</sup>) were below the lower limits of their age-specific reference ranges (0.02x10<sup>9</sup>/L and 0.03x10<sup>9</sup>/L, respectively), while T cell (CD3<sup>+</sup>) numbers were within the normal range (1.27x10<sup>9</sup>/L) with slightly reduced CD4 to CD8 ratio (0.64, later 0.85). "Double negative" CD4<sup>+</sup>CD8<sup>-</sup> T cells were not found in significant numbers, excluding the possibility of autoimmune lymphoproliferative syndrome. This was further confirmed by sequencing the genes for Fas, Fas ligand

**Table I.** HLH-2004 Diagnostic Criteria for Hemophagocytic Lymphohistiocytosis\*

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1. Familial disease/known genetic defect
2. Clinical and laboratory criteria (5/8 criteria required)
Fever
Splenomegaly
Cytopenia $\geq 2$ cell lines
Hemoglobin $<90$ g/L (below 4 weeks $<120$ g/L)
Neutrophils $<1 \times 10^9/L$
Hypertriglyceridemia and/or hypofibrinogenemia
Fasting triglycerides $\geq 3$ mmol/L
Fibrinogen $< 1.5$ g/L
Ferritin $\geq 500$ $\mu\text{g/L}$
sIL-2R $\geq 2400$ IU/ml
Decreased or absent NK-cell activity
Hemophagocytosis in bone marrow, CSF and/or lymph nodes

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Supportive evidence includes cerebral symptoms with moderate pleocytosis and/or elevated protein, elevated transaminases and bilirubin, LDH  $> 1000$  U/l.

\*Henter JI, Horne A, Aricó M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer* 2007; 48: 124-131

and caspase 8, whereby no mutations were found. Bone marrow puncture was performed. The marrow was hypocellular, and small lymphocytes were seen to predominate. A greater than usual number of macrophages was noted, but without overt hemophagocytosis. Granulocytes and their precursors were exceedingly rare. Subsequent bone marrow biopsy showed slight lymphocytosis in the form of small, sometimes confluent, groups of B cells (CD20<sup>+</sup>) or T cells (CD3<sup>+</sup>) with mildly increased numbers of macrophages (CD68<sup>+</sup>), but no overt signs of Langerhans cell histiocytosis (CD1a<sup>+</sup> cells were absent). There were also no signs of hemophagocytosis or lymphoproliferative disease. A cytogenetic diepoxybutane (DEB) test was performed. The karyotype was shown to be normal (46, XX), and chromosomal instability was not found. The plasma concentration of soluble interleukin-2 receptor (sIL-2R) was found to be increased (2771 IU/ml). The patient thus fulfilled five of the eight diagnostic criteria required to establish the existence of secondary hemophagocytic lymphohistiocytosis (Table I) – fever, splenomegaly, pancytopenia, ferritin level  $> 500$   $\mu\text{g/L}$  and sIL-2R  $> 2400$  IU/ml. It is notable that the fibrinogen level was at no

point below the reference range, nor was there an increase in serum triglyceride levels. NK cell activity was not analyzed due to temporary unavailability of a <sup>51</sup>Cr-equipped laboratory facility. The child was treated according to the HLH-2004 protocol for secondary HLH (dexamethasone 10 mg/m<sup>2</sup> daily for 2 weeks, then 5 mg/m<sup>2</sup> for 2 weeks, then 2.5 mg/m<sup>2</sup> for 2 weeks, then 1.25 mg/m<sup>2</sup> for a week; cyclosporine 6 mg/kg daily divided in two doses – later corrected, aiming at a plasma level of 200  $\mu\text{g/l}$ ; but no VP16 as for primary HLH). This treatment was successful in bringing about the regression of splenomegaly as well as normalization of laboratory test results within two months. The child was subsequently kept on cyclosporine maintenance therapy.

At the time of reevaluation upon cessation of treatment (age 5 years 2 months), pediatric physical examination findings were normal. A slight asymmetry of the mandible was still visible. All laboratory parameters were within the reference range, including urea, creatinin and transaminases. The liver and spleen were no longer enlarged, nor were there any enlarged lymph nodes. The child is due to undergo follow-up examinations at regular intervals

for her insipid diabetes, but is otherwise in good health.

### Discussion

Our patient presented with a rash, a feature present in over half of LCH patients<sup>12</sup>. However, this circumstance led to her being treated for LCH by dermatologists. Since LCH is a rare and underdiagnosed disease, treatment in settings other than tertiary care pediatric hematology/oncology centers tends to be incomplete (in the view of the Protocol), and this was the case with our patient. The child did not fall in the high-risk category under the LCH-III Protocol<sup>13</sup>. She tolerated the treatment well, but did develop insipid diabetes – a very frequent manifestation of LCH<sup>14,15</sup>.

The fact that the child was diagnosed with LCH might easily have led physicians to overlook the developing secondary HLH and/or attribute its symptoms and signs to the underlying disease. Furthermore, some of the cardinal symptoms and signs of HLH, such as cytopenias and hepatosplenomegaly, are also features of LCH. However, one should always bear in mind that secondary HLH may arise out of a wide variety of infectious, autoimmune or neoplastic disorders. LCH as the basis for development of HLH, according to an analysis of a large series of clinical cases performed by Favara *et al.*<sup>16</sup>, may be more common than is usually thought. HLH arising out of LCH has also been reported in two patients belonging to case series from Thailand and Turkey<sup>10,17</sup>. Recurrent virus-triggered secondary HLH in a child suffering from LCH has also been reported<sup>18</sup>. It is important to note that the data of Favara *et al.*<sup>16</sup> demonstrate that overt HLH is more frequent in patients with severe forms of LCH. It is, thus, possible that the respective pathogenetic pathways of these two histiocytic disorders partially overlap.

In our previously published HLH case series<sup>19</sup>, six out of 13 children clearly had secondary HLH, while the remaining seven were strongly suspected to suffer from the primary form of the disorder. Our sample is of insufficient size to allow statistical comparisons with series published elsewhere. Furthermore, the incidence of HLH is thought to be seriously underestimated, and the true incidence of secondary HLH is unknown<sup>8,20</sup>. A study

conducted in Turkey by Gürgey *et al.*<sup>21</sup> found that the prevalence of secondary HLH among hospitalized pediatric patients was 0.05%.

Ultimately, it is far from certain that a sharp distinction between primary and secondary HLH is meaningful. Viewed in this light, the possibility that our patient had some degree of genetic predisposition that increased the likelihood of occurrence of both disorders—in accordance with the proposed hypothesis that secondary HLH is also a consequence of various unknown genetic factors, acting in concert with another primary disorder that sets the chain of pathologic events in motion by activating the cytokine cascades that stimulate macrophages—can by no means be excluded<sup>22</sup>. It is thus possible that, in the future, “primary” and “secondary” HLH will be regarded less as absolutely distinct nosological entities and more as two opposing ends of a continuum.

### Conclusion

The potential presence of HLH should always be investigated in a child suffering from hepatosplenomegaly and cytopenias, with or without a known primary disorder. It may be worthwhile to perform the laboratory tests that establish the diagnostic criteria for HLH, even if the latter is suspected as a “second histiocytosis” in a child already diagnosed with a histiocytic disorder, such as LCH.

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