Familial hemophagocytic lymphohistiocytosis: how late can the onset be?

Michaela Allen,* Carmela De Fusco,° Faezeh Legrand,# Rita Clementi,@ Valentino Conter,^ Cesare Danesino,@ Gritta Janka,§ Maurizio Aricò*

*Department of Pediatrics, University, IRCCS Policlinico San Matteo Pavia, Italy; °Department of Pediatrics, Ospedale Pausilipon, Napoli, Italy; #Service d'Hemato-Immunologie, Hopital R.Debrè, Paris, France; @Biologia Generale e Genetica Medica, Università di Pavia, Italy; ^Department of Pediatrics, Ospedale S.Gerardo, Monza, Italy; §Department of Pediatric Hematology and Oncology, University Childrens Hospital, Hamburg, Germany

Background and Objectives. Most patients with familial hemophagocytic lymphohistiocytosis (HLH) develop the disease within the first two years of age. In a minority of cases a later occurrence has been reported, with an upper age limit of eight years. A significant concordance of the age at onset within each family has also been observed.

Results. We report four cases of families with HLH diagnosed at an unusually late age, comprised between between 9 and 17 years; in each of these families another child developed the disease in infancy. The natural killer activity of the patients was depleted; nevertheless, we had indirect evidence that, in at least two families, mutations of the perforin gene were not causing the disease.

Interpretation and Conclusions. Such a late onset is very unusual and suggests that there is a subgroup of families with HLH in which the disease may present early or late in different members. Thus in some families with HLH the siblings might remain at risk of developing the disease for several years. Their actual risk cannot be defined until the genetic mutation is identified in each family and assessed in each member.

Key words: Lymphohistiocytosis, late onset, perforin, hemophagocytosis

original paper

haematologica 2001; 86:499-503
http://www.haematologica.it/2001_05/0499.htm

Correspondence: Maurizio Aricò, M.D., Clinica Pediatrica, IRCCS Policlinico San Matteo, 27100 Pavia, Italy.
Phone: international +39-0382-502916 – Fax: international +39-0382-502601 – E - mail: aricom@unipv.it

Hemophagocytic lymphohistiocytosis (HLH) is a rare, autosomal recessive disorder, with invariably fatal outcome in untreated patients.[1,2] The use of chemotherapy and immunosuppressive drugs will result in temporary disease control but only bone marrow transplantation (BMT) is curative.[3,4] The main features of HLH, which as of 1991 have also become the established criteria for diagnosis, are: a high, persistent or intermittent fever; splenomegaly; cytopenia; hypertriglyceridemia or hypofibrinogenemia; and hemophagocytosis in the bone marrow, spleen or lymph nodes by activated macrophages.[5] Other frequent signs of the disease are hepatomegaly and central nervous system (CNS) involvement. Differential diagnosis from other disorders may still present difficulties. After two regions linked to HLH on chromosomes 9 and 10 were identified,[6,7] Stepp *et al*. found nine different mutations in the perforin 1 gene (*PRF1*, located on chromosome 10) in a group of eight unrelated patients, providing evidence for the first time of a relation between a disease and *PRF1*.[8] Nevertheless, other cases have been reported of families with HLH in whom linkage to both regions was excluded, suggesting extensive heterogeneity.[9-11]

HLH is known to affect mainly children in early infancy. The peak age at diagnosis is between one and six months. Exceptions to this general rule have been observed, as there have been reports of familial cases with an age at onset of up to eight years.[2,12] Furthermore it has been observed that the age at onset between affected siblings is usually comparable, and quite often coincides.[2]

We have recently come across four families with a child who developed HLH in early infancy and whose sibling was diagnosed as having the same disease at

an age that up till now would have been considered *safe*, i.e. 17, 12, 12.2 and 24 years. The main features of the four patients and their families are summarized in Table 1.

The aim of this report is to increase awareness on the possibility that HLH may have a late onset in some families, an event which may have strong implications regarding genetic counseling and the selection of BMT donors.

Design and Methods

Case #1

Our patient was the second of three brothers born to apparently healthy consanguineous parents (first cousins) of southern Italian origin. His family history revealed that four of his father's brothers and sisters died in infancy of undefined diseases. His firstborn and eldest brother, was admitted to the local hospital with intermittent high fever, purpura, hepatosplenomegaly, cervical lymphoadenopathy, anemia and moderate thrombocytopenia, at the age of 13 months. At the time, infectious mononucleosis was suggested as the cause of his illness. He died five months later of progressive disease which included CNS deterioration. *Lymphoproliferative disease with meningo-encephalitis* was the reported cause of death.

Our patient had always been healthy except for a mild thrombocytopenia repeatedly observed on routine tests. At seventeen he developed mumps complicated by salivary adenitis, epididymo-orchitis and pancreatitis treated with low-dose steroids. After three weeks he developed fever, ataxia, diplopia and meningeal irritation associated with mild hepatosplenomegaly, attributed to paramyxovirus encephalitis. When re-examined three months later, the neurological signs where unvaried, his liver and spleen were further enlarged, he had polyadenopathy, pneumonia and high levels of transaminases. As empirical anti-tuberculosis therapy gave no improvement, a lymph node biopsy was obtained, showing *reactive histiocytosis of the lymph node sinus*. Tuberculosis and other bacterial infections were excluded. His clinical course was characterized by several reactivations, complicated first by hydrocephalus, then by sepsis due to *Xanthomonas maltophilia*. As his conditions were constantly worsening, with progressive cytopenia, deteriorating liver function, hyperferritenemia, hypertriglyceridemia, hypofibrinogenemia and CSF pleocytosis (20/mm^3), a bone marrow aspirate was taken, which showed a normal picture except for an increase of macrophages engaged in hemophagocytosis. This last finding, together with his clinical and laboratory features and the knowledge of his younger brother's history, finally led to the diagnosis of HLH. His NK cells' activity proved to be totally impaired at the diagnosis and one month later. Analysis of polymorphic markers closely located to *PRF1* (D10S537, D10S676) demonstrated that the patient was heterozygous for both loci. This result, together with the information on his parents' consanguinity, makes mutation of *PRF1* gene highly unlikely. As EBNA and VCA-EBV antibodies were observed and his family history was compatible with X-linked lymphoproliferative disease, the sequences of the four SH2D1A gene exons were analyzed showing no mutation. Unfortunately, despite a good initial response to treatment with etoposide and dexamethasone according to the HLH-94 protocol,[3] he died of sepsis, aged 18.8 years. His third brother is still completely healthy at the age of 16.

Case #2

The patient, female, twelve years of age, was the third child born to healthy consanguineous parents (second cousins) of southern Italian origin. The family history revealed that her oldest sister fell ill at seven months with recurrent bouts of high fever lasting a week each, accompanied by increasing pallor and hepatosplenomegaly. Laboratory tests showed severe anemia and thrombocytopenia, and negative direct and indirect Coombs tests, while no infection could be proved: Vidal-Wright and Paul-Bunnel tests, blood and urine cultures and fecal parasitology tests were all negative. Both bone marrow aspirates obtained showed a picture of reduced maturation of the erythroid and granulocyte lines, while eosinophils and lymphocytes were increased in number. She died six months later with a diagnosis of *reticuloendotheliosis*.

Our patient was admitted to hospital with high fever, pancytopenia, hepatosplenomegaly, headache and signs of meningeal irritation, a clinical picture that closely resembled that of her sister despite the age difference. Laboratory tests showed high serum levels of triglycerides, ferritin and transaminases, CSF pleocytosis and hemophagocytosis in the bone marrow, as well as an underlying EBV infection (positive IgM antibodies). Therefore HLH was diagnosed. Her NK activity was totally impaired at two subsequent evaluations, the first at diagnosis and the second during clinical remission. Analysis of polymorphic markers closely located to the *PRF1* gene (D10S537, D10S676) demonstrated that the patient was heterozygous for both loci, a result which is not consistent with a mutation of this gene. She underwent initial therapy according to the HLH-94 protocol, with

excellent initial disease control; transient treatment withdrawal was followed by a reactivation which is presently being controlled by therapy, 6 months from diagnosis, while awaiting BMT. Her brother is still healthy at the age of sixteen.

Case #3

The patient is a 12.2 year-old male born to healthy consanguineous parents (first degree cousins) of Iranian origin, who had previously lost a daughter in early infancy, and have a 9-year old healthy son. The affected sister died at the age of 22 months after developing the following clinical picture: 40°C fever, maculopapular skin rash, hepatosplenomegaly, diffuse lymphadenopathy, pulmonary distress and high WBC count (45,000/mm^3). The autopsy performed in Iran suggested a monocytic leukemia or a lymphoproliferative syndrome as the probable cause of death. Hepatic and lymph node biopsies were reviewed years later and interpreted as signs of a lymphoproliferative disease with macrophage activation visible in the lymph node section.

Our patient was first admitted to hospital in Tehran for a sudden reduction of eyesight. Chorioretinitis localized in his right eye was diagnosed and explained by positive serology for toxoplasma. After five weeks of treatment with steroids and cotrimoxasole, fever and cytopenia set in. A bone marrow aspirate revealed a hypocellular marrow. In the next few weeks his medical history was characterized by recurrent bouts of fever accompanied by progressive cytopenia. During a visit to France three months after the onset of the first symptoms, he was taken to the Robert Debré Hospital in Paris. Clinical and laboratory findings revealed a picture very similar to that of his younger sister: very high fever, generalized edema, hepatosplenomegaly, cytopenia, CSF pleocytosis (49/mm^3) and macrophage activation syndrome with hyperferritenemia, hypertriglyceridemia, severe hypofibrinogenemia and signs of hepatic cytolysis; no response to antibiotic therapy was observed. A screening for various infectious agents (CMV, EBV, HSV, adenovirus, HAV, HCV, toxoplasma, malaria, leishmania) did not explain the clinical picture, whereas a second bone marrow aspirate showed various images of hemophagocytosis. Because all the diagnostic criteria were fulfilled, including a positive family history, a diagnosis of familial HLH was established, with at least three documented reactivations; NK activity was tested at the time of diagnosis and found depleted. Perforin expression evaluated by confocal microscopy, was normal. This finding, together with the results of linkage analysis, ruled out a *PRF1* gene mutation. Treatment with cyclosporin A, steroids, and intrathecal methotrexate was started with immediate and persistent response. The child is currently awaiting a BMT from a matched unrelated donor, eighteen months after the diagnosis.

Case #4

The patient is the second son of unrelated healthy parents. An older brother died at three years of age after an eleven-month illness with repeated episodes of fever, hepatosplenomegaly, jaundice, anemia and elevated cells and protein in the CSF. A younger brother died at six years after a two-year history of repeated episodes of fever, hepatosplenomegaly, anemia, leukopenia and jaundice. Erythrophagocytosis was found in the bone marrow and the spleen and a diagnosis of HLH was made. He died of CNS involvement.

The patient had a first transient episode of fever, jaundice and thrombocytopenia at nine years of age. From 14 years of age he had repeated episodes of fever, hepatosplenomegaly, moderate anemia and leukopenia, thrombocytopenia and marked hyperbilirubinemia with values of up to 45 mg/dL (mostly unconjugated). Triglycerides were elevated and fibrinogen decreased on a couple of occasions. At sixteen increased histiocytes with erythrophagocytosis were found in the bone marrow. The patient was treated with plasmapheresis and corticosteroids. At twenty-three years old he had his first neurological symptoms with headaches, visual problems, loss of sensory and motor functions, and later, seizures. CSF cells, protein and neopterin were elevated; histiocytes with erythrophagocytosis were found. Magnetic resonance imaging showed areas of demyelination. The bone marrow again showed increased histiocytes with erythrophagocytosis. He was treated with etoposide, corticosteroids and cyclosporin A. At twenty-four years he had persistent hyperbilirubinemia and moderate thrombocytopenia. A negative NK cell activity was found, confirming the previous suspicion of familial HLH. Workup for infections or metabolic diseases was negative. Repeated tests of NK activity while the patient had only CNS manifestations confirmed complete depletion. The patient suffered from a cerebral hemorrhage but on recovery underwent a successful transplantation from his HLA-identical brother at the age of twenty-four. He is now thirty years old and has residual visual problems and epilepsy.

Discussion

We describe four families with children who developed HLH at an unusually late age (Table 1). Since a specific marker of the disease is not available for all patients, the diagnosis of HLH still relies on clinical grounds. Even the recently available genetic marker,

Table 1. Main features in patients with HLH and late onset.

	Case 1	*Case 2*	*Case 3*	*Case 4*
Consanguinity	Yes	Yes	Yes	No
Geographic origin	South Italy (Campania)	South Italy (Campania)	Iran	Germany
Number of sibs/affected	2/1	2/1	2/1	3/2
Age at onset in affected sibs (mos.)	13	7	22	25 and 48
Gender	M	F	M	M
Age at onset	17 yrs	12 yrs	12.2 yrs	9 yrs
Age at diagnosis	18 yrs	12 yrs	12.5 yrs	24 yrs
Fever	+	+	+	+
Hepatosplenomegaly	+	+	+	+
Skin rash	-	-	-	-
Lymph nodes	+	-	-	-
CNS involvement	-	+	+	+
Edema	-	-	+	-
Triggering infection	Paramyxovirus	EBV	Toxoplasma	-
Jaundice	-	-	-	+
Anemia (<9 g/dL)	+	+	+	+
Thrombocytopenia (100,000/mm^3)	+	+	+	+
Neutropenia (<1000/mm^3)	+	+	+	-
Hypertriglyceridemia (>2 mmol/L)	+	+	+	+
Hypofibrinogenemia (<150 mg/dl)	-	-	+	+
CSF pleocytosis	+	+	+	+
Hyperferritinemia	+	+	+	-
Hypertransaminasemia	+	+	+	+
Hyponatremia	not tested	-	+	-
Hypoalbuminemia	not tested	-	+	-
Hemophagocytosis	+	+	+	+
Response to therapy	+	+	+	+
State or FUP	Dead of sepsis during therapy	Alive in R, on therapy	Alive in R, on therapy	Alive after BMT
NK activity at E:T ratios 10:1,30:1,100:1	**0, 0, 4**	**0, 0, 0**	**4, 5, 5**	**0, 0, 0**
Analysis of SH2D1A gene	wild type	not applicable	not applicable	not done
Linkage to 10q21-22	unlikely	unlikely	excluded	not done
Perforin Expression	not done	normal	normal	not done

Items in bold are established diagnostic criteria for HLH (Ref.5); SH2D1A: gene involved in X-linked lymphoproliferative disease (XLP). R = remission.

i.e. *PRF1* gene mutation,[8] does not account for all cases, and is not easily available in all patients. Perforin protein is necessary for the integrity of natural killer (NK) activity. In our four families, NK activity was repeatedly tested and found persistently depleted, suggesting a constitutional defect.[13,14] Nevertheless, the disease was not associated with *PRF1* gene mutations at least in two of them, as indicated by fully normal perforin expression and by the results of analysis of microsatellites flanking the gene. This confirms the heterogeneity of HLH, which might be caused by mutations in genes other than *PRF1*, involved in NK function.

Preliminary observations in patients of Italian, Ghanaian and Turkish origin, suggest that HLH due to mutation in the *PRF1* gene is associated with a very early onset, comparable in all the affected members of a family.[9] The four families we describe prove that when the disease occurs as a consequence of mutations in one or more different genes, other than *PRF1*, involved in NK function, for unknown reasons a delayed onset is possible.

The age at onset does not seem to affect the course of the disease which may end up being equally aggressive and life-threatening. The observation that the younger patients from the same families apparently had a more aggressive course could partly be explained by more specific treatment administered in the index cases. The HLH-94 protocol, consisting in the combined use of etoposide, dexamethasone and cyclosporine, has proved to be very effective in achieving initial disease control, and, although not curative, it allows more patients to progress to BMT (*Henter and Aricò, personal communication, 2001*).

Evidence of infections at the time of the diagnosis of HLH is common. Whether the triggering infections, either viral or parasitic, observed in three of the patients with late onset, might have played a fundamental role in breaking the equilibrium of the immune system remains questionable.

We suspect that the cases described are part of a different subset of HLH. Interestingly, in the two Italian families, patients had a common geographic origin, as their families came from two very small villages close to each other. In a recent epidemiologic study, the whole region of Campania, where the two Italian families came from, turned out to be one of the two hot spots for HLH in Italy.[15]

In the absence of a specific marker, clinical and genetic counseling to families with HLH may present difficulties. In particular, assessment of asymptomatic siblings is not easy. For this purpose the information that the age at onset of the disease is often homogeneous within each family, derived from the HLH Registry study,[2] has been used up to now by clinicians to reassure the parents that their older children are not likely to develop the disease. This procedure has some obvious limitations, including the risk of erroneously selecting an asymptomatic sibling as a donor for bone marrow transplantation.[16] The cases presented support the concept that the age criteria is not always reliable for such surrogate genetic counseling. Genetic studies should be undertaken in all

available cases to assess the PRF1 gene status; in patients without mutations of PRF1, research aimed at identifying new genes should be pursued. In the meanwhile, the hypothesis of using impaired NK activity as a surrogate marker of the disease, especially in the evaluation of familial donors, is being explored.[17]

Contributions and Acknowledgments

MA was the key person in the project for data collection and preparation of the manuscript. CDF, EV, VC, GJ and MA identified and cared for the patients. CD, RC were responsible for the genetic study. MA: principal investigator of the HLH project, was responsible for conception of the study, analysis and interpretation of data; he also supervised the drafting of the article. All the authors gave their final approval of the version to be published.

Funding

This work was supported in part by the following grants: Telethon Italy, Grant C30 (CD) and E755 (MA); Ricerca Corrente 390RCR97/01 and 80291, IRCCS Policlinico San Matteo, Pavia, Italy (MA); and by the "Associazione Antonio Pinzino" (Petralia, Palermo, Italy).

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Executive Editor. The final decision to accept this paper was taken jointly by Prof. Cazzola and the Editors. Manuscript received February 19, 2001; accepted Marfch 28, 2001.

Potential implications for clinical practice

These data expand the age span in which the differential diagnosis of HLH should be considered.

References

1. Farquhar J, Claireaux A. Familial haemophagocytic reticulosis. Arch Dis Child 1952; 27:519-25.
2. Aricò M, Janka G, Fischer A, et al. Hemophagocytic lymphohistiocytosis. Report of 122 children from the International Registry. FHL Study Group of the Histiocyte Society. Leukemia 1996; 10:197-203.
3. Henter JI, Aricò M, Egeler RM, et al. HLH-94: a treatment protocol for hemophagocytic lymphohistiocytosis. HLH study Group of the Histiocyte Society. Med Pediatr Oncol 1997; 28:342-7.
4. Jabado N, de Graeff-Meeder ER, Cavazzana-Calvo M, et al. Treatment of familial hemophagocytic lymphohistiocytosis with bone marrow transplantation from HLA genetically nonidentical donors. Blood 1997; 90:4743-8.
5. Henter JI, Elinder G, Ost A. Diagnostic guidelines for hemophagocytic lymphohistiocytosis. The FHL Study Group of the Histiocyte Society. Semin Oncol 1991; 18: 29-33.
6. Ohadi M, Lalloz MR, Sham P, et al. Localization of a gene for familial hemophagocytic lymphohistiocytosis at chromosome 9q21.3-22 by homozygosity mapping. Am J Hum Genet 1999; 64:165-71.
7. Dufourcq-Lagelouse R, Jabado N, Le Deist F, et al. Linkage of familial hemophagocytic lymphohistiocytosis to 10q21-22 and evidence for heterogeneity. Am J Hum Genet 1999; 64:172-9.
8. Stepp SE, Dufourcq-Lagelouse R, Le Deist F, et al. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. Science 1999; 286:1957-9.
9. Clementi R, zur Stadt U, Janka G, et al. Six novel mutations in the PRF1 gene in children with hemophagocytic lymphohistiocytosis. J Med Genet, in press.
10. Graham GE, Graham LM, Bridge PJ, et al. Further evidence for genetic heterogeneity in familial hemophagocytic lymphohistiocytosis (FHLH). Pediatr Res 2000; 48:227-32.
11. zur Stadt U, Kabisch H, Janka G, Schneider M. Mutational analysis of the perforin gene and NK-cell function in hemophagocytic lymphohistiocytosis. Proceedings of the Histiocyte Society 16th Annual Meeting. Amsterdam 2000, September 29-October 2.
12. Mozziconacci P, Nezelof C, Mozziconacci P, et al. La lymphohistiocytose familiare. Arch Fr Pediatr 1965; 22:385-408.
13. Perez N, Virelizier JL, Arenzana-Seisdedos F, Fischer A, Griscelli C. Impaired natural killer activity in lymphohistiocytosis syndrome. J Pediatr 1984; 104:569-73.
14. Aricò M, Nespoli L, Maccario R, et al. Natural cytotoxicity impairment in familial haemophagocytic lymphohistiocytosis. Arch Dis Child 1988; 63:292-6.
15. Aricò M, Fiorani O, Lis A, et al. Incidence and geographic distribution of HLH in Italy. Proceedings of the Annual Meeting of the Histiocyte Society, Amsterdam 2000, September 29- October 2, 42.
16. Blanche S, Caniglia M, Girault D, Landman J, Griscelli C, Fischer A. Treatment of hemophagocytic lymphohistiocytosis with chemotherapy and bone marrow transplantation: a single-center study of 22 cases. Blood 1991; 78:51-4.
17. Sullivan KE, Delaat CA, Douglas SD, Filipovich AH. Defective natural killer cell function in patients with hemophagocytic lymphohistiocytosis and in first degree relatives. Pediatr Res 1998; 44:465-8.