Haematologica 1998; 83:347-349 decision making and problem solving

Decision making at the bedside: diagnosis of hereditary spherocytosis in a transfused infant

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ABSTRACT

A large group of hereditary spherocytosis (HS) patients manifest the clinical signs of this condition during the neonatal period and most of them require transfusions. The authors describe a clinical case of a neonate that was transfused. They demonstrated that the splenectomized mother had an HS due to a *de novo* mutation of one ankyrin allele. By means of this molecular approach, they were able to perform a diagnosis of HS in the newborn. The administration of rhEpo during the first months of life created a condition of transfusion-independence and, after six months, they were able to demonstrate the biochemical defect on the red cell membrane.

Key words: spherocytosis, newborn, anemia, red cell membrane, cytoskeleton, rhEpo

Case Report

Step I

Propositus TG was born in 1997 in Naples following a normal pregnancy (weight at term: 3,450 g); he was the first son of a woman who suffered from a mild to moderate hemolytic anemia never requiring transfusions, fully improved after splenectomy performed when she was 13 years old. Before splenectomy, the mother received a diagnosis of elliptocytosis.

TG had neonatal hyperbilirubinemia and underwent phototherapy; two weeks later, as a consequence of pallor and jaundice, he was admitted in a hospital; routine hematological data pointed out a severe anemia (Hb: 45 g/L) with slightly high percentage of reticulocytes (4%) and elevated level of unconjugated bilirubin (5.8 mg/dL). Coombs test was negative. Considering the particularly low level of hemoglobin, the patient, without further diagnostic assessments, was immediately transfused and the day after transferred to our hospital.

Step II

Since TG had been recently transfused, it was not possible to perform any diagnostic evaluation on circulating erythrocytes. For this reason, we decided to intensively investigate the propositus' mother and her family in order to recognize the molecular alteration responsible for the hemolytic anemia. Hypothesizing a dominantly inherited disease, our purpose was, once characterized by the causal mutation, to search for it in the propositus.

Peripheral blood smear observation of the subject II-2 (Figure1) showed anisopoikilocytosis with a striking microspherocytosis; the osmotic fragility of fresh erythrocytes performed with scalar concentrations of sodium chloride as well as Pink test (percentage of hemolysis: 73%) were increased. Both parents (I-1 and I-2) were clinically and hematologically normal. Our hypothesis was that the proband's mother was erroneously classified as elliptocytosis, whereas she had a genuine spherocytosis. To confirm this, we performed a biochemical analysis of rbc membrane proteins. The pattern of these was latter evaluated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), using, with some modifications, both the discontinuous buffer system of Laemmli with acrylamide linear gradient from 5% to 15% and the continuous buffer system of Fairbanks with exponential gradient of acrylamide from 3.5% to 17%. Combined, mild, spectrin and ankyrin deficiency was found (Table 1). The concentration of the other membrane proteins was in the normal range. We made diagnosis of HS due to ankyrin and spectrin deficiency. Considering the normality of both parents, either a recessive manner of transmission or a *de novo* mutation were possible.

Since an HS patient with a *de novo* mutation can be considered the founder of a dominantly inherited HS and consequently, can transmit HS to 50% of his/her offspring, we verified this hypothesis in the subject II-2.

Step III

A number of mutations of the ankyrin gene (ANK1) have been described in dominant HS with combined ankyrin and spectrin deficiency.[1] Many of these are nonsense or frameshift mutations leading to lost expression of the relative alleles. We recently showed that the loss of expression of one ANK1 allele due to *de novo* mutations occurs with an unexpected high frequency (12 out of 19 investigated patients) in subjects with HS, ankyrin and spectrin reduction and normal parents.[2] Therefore, we analyzed the expres-

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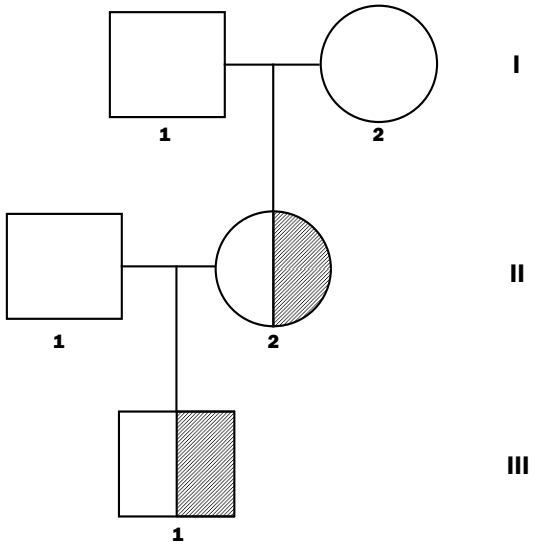

Figure 1. Pedigree of the family affected with HS due to a *de novo* mutation of the ankyrin gene in the subject II-2.

sion of both ANK1 alleles of the propositus' mother (II-2). Genomic DNA was collected from nucleated blood cells. Distribution of the dinucleotide repeat (AC)n polymorphism, located in the non-coding sequence of the last exon of ANK 1 gene, was evaluated by PCR-amplification of the ANK1 gene appropriate segment. Four different sizes of the PCR products may usually be observed, corresponding to the A1, A2, A3 and A4 alleles, characterized by 14, 13, 12 and 11 AC repeats, respectively. Silver stained acrylamide gel showed the subject to be heterozygous for the AC repeat lengths. Total reticulocyte RNA was isolated by the ammonium chloride lysis technique, reversely transcribed into cDNA and PCR amplified using the same conditions as for genomic DNA. Contrasting with the data obtained at the genomic level, II-2 appeared to be homozygous at the cDNA level. This discrepancy strongly indicated the absence of one of the two ankyrin transcriptional messages suggesting the presence of a mutational event (occurred *de novo*) affecting the expression of one ANK1 allele. The same finding was observed in the propositus (III-1), which evidently inherited the mutated ANK1 allele by the mother.

Once established by means of molecular biology, the diagnosis of HS in the propositus, considering that hemoglobin values not sustained by an appropriate reticulocytes count, progressively were falling, rh-Epo was administered at a dose of 500 IU/kg/week.[3]

Step IV

Rh-EPO therapy resulted in an abrupt rise in reticulocyte number, allowing a transfusion independence. After four months, it was possible to analyze erythrocyte membrane of the propositus. As expected by molecular biology results, after normalization of ankyrin concentration on the number of circulating reticulocytes, a combined spectrin and ankyrin deficiency was found (Table 1).

Discussion

HS is a commonly inherited hemolytic anemia due to erythrocyte membrane proteins primitive pathology, characterized by spheroidal shape of red blood cells that have a reduced cell surface area-to-cell volume ratio.[4] These cells are selectively trapped in the spleen and destroyed.

The characteristic clinical findings are anemia, jaundice, splenomegaly and increased red cell osmotic fragility.

HS exhibits both dominant and recessive patterns of inheritance. About 75% of families display an autosomal dominant transmission, whereas in about 25%

Table 1. Clinical, biochemical and molecular data of the family affected with HS due to a *de novo* mutation of the ankyrin gene in the subject II-2.

	I-1	*I-2*	*II-1*	*II-2*	*III-1* 15 days*	*III-1 1 month*	*III-1 2 months*	*III-1 6 months*
Hb (g/dL)	13.2	15.4	12.8	14.7	4.5	7.2	9.1	9.8
Reticulocytes (%)	0.8	0.7	1.1	1.0	4	4.1	10.3	11
Bilirubin (mg/dL)	0.9	0.6	0.9	0.7	3.8	4.2	2.7	2.9
Pink-Test (%)	11	13	73	14	nd	nd	nd	66
Spec (%)	105	98	81	103	nd	nd	nd	83
Ank.(%)	100	103	79	97	nd	nd	nd	81
(CA)nAnk. DNA	14/11	14/11	14/11	14/14	nd	14/11	nd	nd
(CA)nAnk. RNA	14/11	14/11	14/–	14/14	nd	14/–	nd	nd
Transfusions	no	no	no	no	yes	no	no	no
RhEpo	no	no	no	no	no	yes	yes	yes

**These data were obtained before transfusion.*

of the kindred, both parents are clinically normal. These patients could have a genuinely recessive form of HS, but one cannot exclude a dominant HS resulting from a *de novo* mutation.[5]

Clinical spectrum of HS is heterogeneous: mild (20-30%), typical (60-70%) and severe, transfusion dependent forms (5%) have been described. Although during the first days of life, anemia and jaundice are present in about 60% of overall HS subjects.[6]

Approximately 40-45% of neonates with HS are so anemic, requiring one or more transfusions.[7]

Laboratory diagnosis of HS is quite difficult during the neonatal period. Reticulocyte count is often normal or slightly enhanced and haptoglobin level is not a reliable indicator of hemolysis during this period. Furthermore, fetal erythrocytes are more osmotically resistant than adult cells, which should diminish the sensitivity of the unincubated osmotic fragility test. However, incubated osmotic fragility tests are a reliable diagnostic tool in the neonatal period and coupled, when necessary, with the biochemical assay of the cytoskeletal proteins could allow the identification, in our experience, of a good number of cases.[8]

Obviously, both fragility osmotic tests and membrane proteins evaluation cannot be performed when the patient has been transfused. Although analysis of family members may be of help in the HS dominantly inherited patients, diagnosis has to be carried out by means of molecular biology technologies.[9,10]

For a correct assessment of the inheritance patterns, as suggested in the above presented case, molecular study is recommended in the HS infants which have required transfusion before diagnostic classification as well as in HS patients with normal parents.

Due to a bone marrow delayed response to anemia during the first months of life, some HS infants may become progressively more anemic and require transfusions. Fortunately, the problem is transient in the majority of patients and usually is destined to remit. Subsequently, the course of the disease depends on the equilibrium established between the rates of red-cell production and destruction. To reduce the blood requirement during infancy, it has been very recently proposed to treat HS newborns with rH-Epo at a dose of 500-1000 I.U./kg/week. Our case, together with preliminary results of other authors,[3] suggest that treatment with rH-Epo could be an alternative to regular transfusion during the first months of life.

Manuscript processing

Manuscript received November 18, 1997; accepted January 26, 1998.

References

1. Eber SW, Gonzalez JM, Lux ML, et al. Ankyrin-1 mutations are a major cause of dominant and recessive hereditary spherocytosis. Nature Genet 1996; 13:214-8.
2. Miraglia del Giudice E, Francese M, Nobili B, et al. High frequency of *de novo* mutations in ankyrin gene (ANK 1) in children with hereditary spherocytosis. J Pediatr 1998; 132:117-20.
3. Delhommeau F, Cynober T, Mielot F, Schischmanoff PO, Tchernia G. Hereditary spherocytosis during the first year of life. Rh-EPO versus multiple transfusions [abstract]. 2nd Meeting of the EHA Working Group on Red Cell Membrane, Paris, 1996.
4. Lux SE, Palek J. Disorders of red cell membrane. In: Handin RI, Lux SE, Stossel TP, eds. Blood: principles and practice of hematology. Philadelphia: Lippincott Comp; 1995:1701-818.
5. Hassoun H, Palek J. Hereditary spherocytosis: a review of the clinical and molecular aspects of the disease. Blood Rev 1996; 10:129-47.
6. Iolascon A, Miraglia del Giudice E, Perrotta S, Morlé L, Alloisio N, Delaunay J. Hereditary spherocytosis: from clinical to molecular defects. Haematologica 1998; 83:240-57.
7. Pinto L, Iolascon A, Miraglia del Giudice E, Matarese SMR, Nobili B, Perrotta S. The Italian survey on hereditary spherocytosis. Int J Ped Hematol Oncol 1995; 2:43-7.
8. Iolascon A, Perrotta S, Tavano R, Miraglia del Giudice E. Hereditary spherocytosis in newborns. In: Bellanti JA, Bracci R, Prindull G, Xanthou M, eds. Neonatal hematology and Immunology. III. Amsterdam: Elsevier; 1997. p. 197-202.
9. Lanciotti M, Perutelli P, Valetto A, Di Martino D, Mori PG. Ankyrin deficiency is the most common defect in dominant and non dominant hereditary spherocytosis. Haematologica 1997; 82:460-2.
10. Miraglia Del Giudice E, Francese M, Polito R, Nobili B, Iolascon A, Perrotta S. Apparently normal ankyrin content in unsplenectomized hereditary spherocytosis patients with the inactivation of one ankyrin (ANK1) allele. Haematologica 1997; 82:332-3.