Table 2. Erythroid membrane protein defects and clinical course of hereditary spherocytosis.

Defect	Mild HS n=20	p=10	Typical HS n=34	p=14	Severe HS n=8	p=6	Total HS n=62	p=30
Sp↓ Ank N	8 (40%)	5 (50 %)	9 (26.4%)	2 (14.3%)	2 (25%)	1 (16.6%)	19 (30%)	8 (26.6%)
Sp↓ Ank↓	9 (45%)	4 (40%)	19 (55.9%)	10 (71.4%)	6 (75%)	5 (83.3%)	34 (55%)	19 (63.3%)
Sp N Ank N	1 (5%)	1 (10%)	2 (6%)	–	–	–	3 (4.8%)	1 (3.3%)
Sp N Ank↓	2 (10%)	–	2 (6%)	1 (7.1%)	–	–	4 (6.4%)	1 (3.3%)
Sp↑ Ank↑	–	–	2 (6%)	1 (7.1%)	–	–	2 (3.2%)	1 (3.3%)

Mild HS: patients with normal hemoglobin and <200x10⁹/L reticulocytes. Typical HS: cases with episodic anemia, no transfusional needs and >200 x10⁹/L reticulocytes. Severe HS: subjects with serious chronic, hemolytic anemia, improving after splenectomy. Sp: spectrin, Ank: ankyrin, N: normal, n: total number of HS patients, p: number of propositi. Percentages (%) of cases in the subsets in brackets. Non-dominant HS: 7 propositi, 3 with typical and 4 with severe illness; 1 patient with severe HS had isolated spectrin deficiency and 6 cases had combined spectrin and ankyrin deficiencies.

Splenectomized patients had mean contents of 64.4%, and 79.1% for typical and severe HS (53.2% and 74.3% for the propositi). Corrected ankyrin contents were lower in unsplenectomized (mean 0.090, SD 0.044) versus splenectomized (mean 0.134, SD 0.046) cases with the same HS severity (p<0.001) seeming to reflect the drop of reticulocytosis after splenectomy and may suggest overcorrection by the proposed equation.[8]

As in other studies,[2,9,10] combined spectrin and ankyrin deficiency was the most prevalent abnormality in our patients. Spectrin deficiency is usually due to a primary decrease of ankyrin[2,8] and ANK1 gene should be investigated;[3,8] although in some cases association with protein 4.2 partial deficiency have been found.[10] A higher frequency of isolated spectrin deficiency and normal or increased spectrin and ankyrin contents have been reported elsewhere.[4] Our results might be due to variations in relative prevalences and methodological limitations because SDS-PAGE in continuous systems tends to undervalue protein bands. This fact could explain our small number of HS cases with band 3 deficit and the lack of significant differences in protein contents between unsplenectomized and splenectomized patients. SDS-PAGE in discontinuous systems with gradient gels yields a more accurate evaluation of protein bands.

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Key words

Hereditary Spherocytosis, laboratory diagnosis, spectrin deficiency, ankyrin deficiency, prevalence

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Acute Parvovirus B19 infection as a cause of autoimmune hemolytic anemia

A patient with homozygous beta-delta thalassemia developed a severe cold hemagglutinin disease with reticulocytopenia. Acute parvovirus B19 infection was suspected because of the presence of anti-B19 IgM in serum and viral DNA analysis in bone marrow. Evidence of recent B19 infection should be sought in patients with autoimmune hemolytic anemia and reticulocytopenia.

Sir,

Human parvovirus B19 (B19) has been reported rarely as producing autoimmune hemolytic anemia (AIHA).[1-4] In this report, a case of severe AIHA fol-

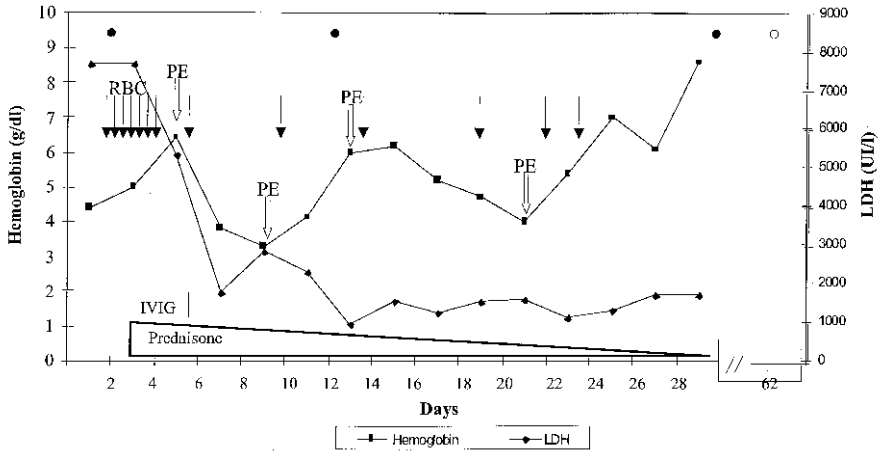

Figure 1. Course of AIHA due to B19 infection. Black arrows indicate red blood cell transfusions (RBC). White arrows indicate plasma exchanges (PE). ● B19-positive IgM; ○ B19-negative IgM.

lowing acute B19 infection is described.

A 5-year old girl with homozygous βδ thalassemia (Hb basal values: 7-8 g/dL) was admitted to our hospital because of a six-day period of fever. She presented with a fine facial rash and petechiae in her armpits and on her palate. Complete blood count showed: Hb 4.3 g/dL, white blood cell count (WBC) 3.6 x10^9/L, platelets 48x10^9/L. Specific IgM and IgG anti-B19 were positive (ELISA), and she was diagnosed as having a transient aplastic crisis due to B19 infection. Shortly thereafter, she was discharged from the hospital in a good clinical state and the following hemogram: WBC 11.2x10^9/L, Hb 7.4 g/dL, platelets 449x10^9/L.

Four days later the patient was readmitted due to the reappearance of fever (40º C) and progressive paleness. Hb value was 4.4 g/dL, with a reticulocyte count of 0.8%, hemoglobinemia (14 g/dL), hemoglobinuria (2.5 g/L), and undetectable serum haptoglobin level. LDH and total bilirubin were 7,663 UI/L and 2.3 mg/dL, respectively. Leukocyte and platelet counts were normal. Bone marrow examination revealed erythroid hyperplasia (myeloid/erythroid ratio 1:8). IgG and IgM anti B-19 antibodies were positive and B19 infection was confirmed by DNA analysis in a bone marrow sample.[5]

Immunohematologic studies showed a positive direct antiglobulin test (IgG^-, $C3d^+$), and a positive antibody screening test (titer <1:1024).[8,9] When cooled at 4º C serum strongly agglutinated test cells (titer: >1:2,056), with agglutination persisting at 30ºC (titer: 1:64). The antibody disappeared after treating the serum with dithithreitol, suggesting it was IgM. Studies with antibodies against I, i, P, and Pr antigens showed no specificity. The eluate, Donath-Landsteiner and *in vitro* test for monophasic acid hemolysins yielded negative results.

Treatment with prednisone, intravenous immune globulin and transfusion of warmed RBCs was started immediately. However, hemolytic activity persisted (Hb value of 3.7 g/dL), and four plasma exchanges were then performed. Transfusion requirements were reduced, hemoglobinemia disappeared, and LDH levels decreased (1,500 IU/L). Three weeks after the last plasma exchange the hemoglobin level returned to baseline values (7-8 g/dL). The girl's clinical course is shown in Figure 1.

B19 uses the high-frequency red cell P antigen as a specific receptor for infection, leading to several hematologic manifestations.[6,7] However, AIHA has been rarely observed; only four similar cases are reported (Table 1).[1-4] Unlike the previous cases, the B19 infection in our case was diagnosed based on the demonstration of viral DNA in bone marrow. Likewise, although clinical manifestations were similar to the previously reported cases, the clinical course in this child was more aggressive. The patient also had reticulocytopenia, but her bone marrow showed erythroid hyperplasia, suggesting that the antibody had some effect on maturing red-cell precursors, preventing their extrusion into the circula-

Table 1. Autoimmune hemolytic anemia due to acute B19 infection.

	Bertrand3	*Smith4*	*Chambers5*	*Chitnavis6*	*This case*
Age (years)/Sex	12/Male	11/Female	3/Male	54/Female	5/Female
Hematologic disorder	None	None	None	None	Homozygous βδ thalassemia
Hemoglobin (g/dL)	6	6.5	5.5	3.1	4.4
Erythroid hypoplasia	Yes	Yes	Yes	Yes	No
Reticulocyte count	Low	Low	Low	Low	Low
B19 diagnosis	B19 IgM	B19 IgM	B19 IgM	B19 IgM	B19 IgM+DNA
DAT	IgG^+	IgG^+ $C3d^+$	IgG^+ $C3d^+$	NA	IgG^- $C3d^+$
Anti-RBC antibody in serum	IgG	IgG Donath-Landsteiner		IgM	IgM
Treatment	RBC transfusion	Steroids	Steroids IVIG RBC transfusion	RBC transfusion Steroids	Steroids IVIG RBC transfusion PE
Status	Alive	Alive	Alive	Alive	Alive

Abbreviations: DAT: Direct antiglobulin test; IVIG: Intravenous immunoglobulin; NA: Not available; RBC: Red blood cell; PE: Plasma exchange.

tion.[10] Nevertheless, it is also possible that the bone marrow examination actually showed relative erythroid hypoplasia. Many patients with homozygous thalassemia have intense erythropoietic marrow activity, with M:E ratios of 1:20. Thus, in this case, a M:E ratio of 1:8 may not truly reflect hyperplasia relative to this child's baseline condition.

Due to the different results reported in the immunohematologic studies, a definite explanation about the mechanism of B19-AIHA is still lacking. Some authors have reported a positive Donath-Landsteiner test after B19-AIHA.[5] This was not found in our patient.

We believe that evidence of acute B19 infection should be sought in patients with AIHA, particularly when associated with reticulocytopenia. Likewise, B19 should be listed among the viral illness associated with acute AIHA.

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Hypereosinophilia associated with dysplastic features and a constitutional translocation previously not described

We report an uncommon case of hypereosinophilia and splenomegaly in a young patient who showed marked morphologic and cytochemical dysplasia of eosinophils together with myeloproliferative and myelodysplastic traits. The patient bore a constitutional translocation not previously described.

Sir,

Many cytogenetic abnormalities have been described in patients with eosinophilia.[1,5] It has been rarely associated with constitutional translocations.[2]

In 1997, Brito-Babapulle published a review on hypereosinophil syndromes and clonal eosinophil disorders which clarified the differential diagnosis between them and establish criteria for the diagnosis of chronic eosinophilic leukemia (CEL). These are: mature eosinophils in peripheral blood, established clonality, absence of dysplastic morphological features, absence of Ph^+ /bcr-abl rearranged cells, absence of clonality.[3]

Nevertheless, the case of one of our patients suggests that these questions are not yet totally clarified. This patient probably suffers from a myeloproliferative disorder with trilineage myelodysplasia and atypical eosinophil proliferation together with a constitutional cytogenetic alteration not previously described.[1,2,5,6]

A 38-years old male with a normal phenotype was referred to our center because of large asymptomatic splenomegaly. His blood count was 9,400 WBC/μL(10% eosinophils, 1% basophils, 3% metamyelocytes, 9% bands, 58% neutrophils, 15% lymphocytes; 4% monocytes), Hb and platelets were within normal limits. A month later, the blood count was 4800 WBC/μL (23% eosinophils and the rest of the differential count similar to the previous one).Biochemical parameters, serology, and iron balance were normal. LAP score was 82 (normal). A bone marrow aspirate showed hypercellularity with 45% of eosinophils in different stages of maturation with numerous mitoses and marked morphologic abnormalities of eosinophils (ringed nuclei, nuclear hypo- and hypersegmentation, cytoplasmic vacuolization), dyserythropoiesis and dysthrombopoiesis. Cytochemical studies: eosinophils were PAS and chloroacetate esterase positive; 2% of ringed sideroblasts. A bone marrow biopsy was hypercellular with an increase of myeloid precursors and a great number of clusters of eosinophils.There were also two clusters of blasts and diffuse reticulin fibrosis.

The karyotype in bone marrow was 46 XY der(8) t(1;8)(p22;q24), and was identical in peripheral lymphocytes stimulated with PHA. Bcr-abl rearrangement