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CLINICAL

PATHOLOGY ROUNDS

Detecting and Identifying Hereditary Pyropoikilocytosis

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Case Presentation

The patient, a 14-year-old adolescent male, was born with neonatal jaundice. A peripheral blood smear revealed numerous abnormalities in RBC morphology. The patient's mother, brother, and maternal grandfather had been previously diagnosed with hereditary elliptocytosis (HE). The patient was subsequently diagnosed with the same disorder.

Since birth, the patient has undergone several hemolytic and aplastic crises that required blood transfusions. At the time of this presentation, he had sought medical attention for evaluation of right upper quadrant pain. Hematology data (Table 1) showed a low hematocrit, mean corpuscular volume (MCV), and hemoglobin level. The red cell distribution width (RDW) was elevated. The peripheral blood smear (Fig 1) disclosed a pronounced number of elliptocytes, spherocytes, tear cells, and poikilospherocytes. A sonogram revealed the presence of gallstones, and the patient was diagnosed with cholecystitis. The symptomatic cholelithiasis was determined to be secondary to increased RBC destruction.

Table 1. Hematology Results for Case Study Patient*

Test	Result	Reference Range
WBC count, × 10^9/L	7.2	5.0–10.0
RBC count, × 10^{12}/L	4.1	4.2–6.0
Hemoglobin, g/L	108	140–180
Hematocrit	0.292	0.400–0.540
MCV, fL	71.9	80.0–96.0
MCH, pg	26.6	26.0–34.0
MCHC, %	37.0	32.0–36.0
Platelet count, × 10^9/L	504	150–400
RDW, %	40.7	11.5–14.5
WBC differential, %		
Segmented neutrophils	50	40–60
Lymphocytes	39	20–40
Monocytes	4	4–8
Eosinophils	7	1–3
Reticulocyte count, %	8.3	0.5–1.5
RBC morphology	Excessive elliptocytes, spherocytes, tear cells; moderate micropoikilospherocytes; occasional Howell-Jolly body	

*SOURCE: Wichita Clinic, Wichita, Kan.

MCV indicates mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width.

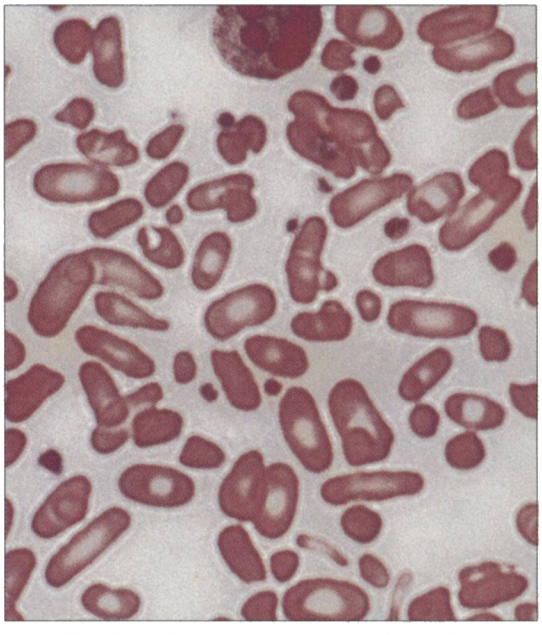

Fig 1. Peripheral blood smear from a patient with hereditary pyropoikilocytosis. Numerous elliptocytes and a moderate number of spherocytes and micropoikilospherocytes are shown (Wright stain, original magnification, ×1,000). Courtesy Elliot Magidson, MD.

The majority of patients diagnosed with HE has few hematologic abnormalities. Although RBC survival time in a patient with common HE may be decreased, hemolysis, if present, is mild and well compensated for by the bone marrow, resulting in the absence of anemia.

In contrast, patients with hereditary pyropoikilocytosis (HPP) usually present with severe hemolytic anemia at birth. The resulting hyperbilirubinemia generally requires phototherapy or exchange transfusion within a short time. The chronic hemolytic state of HPP prolongs the hyperbilirubinemia, and this may lead to the formation of bile stones and subsequent cholecystitis. Because the patient described did not demonstrate the typical clinical features of HE and the peripheral blood smear findings were more consistent with HPP, further laboratory tests were performed.

An osmotic fragility test showed the patient's RBCs to be abnormally susceptible to hemolysis with hypotonic sodium chloride solution (Table 2). A thermal sensitivity test, performed by incubating the patient's blood sample for 10 minutes at 46°C, resulted in marked elongation and fragmentation of the RBCs (Fig 2). Patients whose RBCs show such marked sensitivity are classified as having HPP.[1,2]

To demonstrate differences in thermal stability of RBCs in common HE *vs* HPP, RBCs from the patient's mother were incubated at 46°C. The mother's peripheral blood smear before incubation is shown in Figure 3. After 10 minutes, the mother's RBCs demonstrated only a slight alteration in shape (Fig 4).

On the basis of these results, the patient's condition was reclassified as HPP. His gall bladder was subsequently removed by surgery. The patient recovered quickly and resumed normal activity within 2 months.

Clinical Background

The rare HPP condition is characterized by a defect in the RBC membrane. Although the disorder has been considered a separate entity, many researchers cite convincing evidence that HPP is a subtype,[2] or "aggravated" form of HE.

Both HE and HPP are associated with defects—vertical or horizontal[3]—in protein and lipid interactions within the RBC membrane. Vertical defects involve abnormal interactions between the RBC skeletal lattice proteins and the membrane integral proteins and lipids, causing a loss of membrane surface area and the formation of spherocytes.[1] Horizontal defects concern both abnormal head-to-head

Table 2. Osmotic Fragility Test Results

	Patient	Normal Control
Initial hemolysis	0.60% NaCl	0.45% NaCl
Complete hemolysis	0.35% NaCl	0.30% NaCl

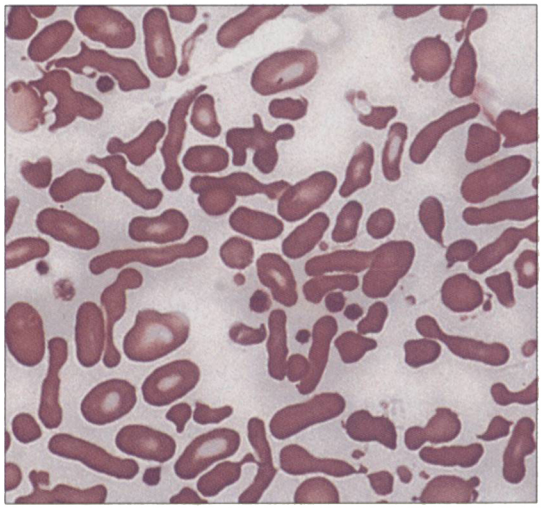

Fig 2. Peripheral blood smear from same patient as in Fig 1, after heating blood to 46°C. Extreme elongation and fragmentation of the RBCs are shown (Wright stain, original magnification, ×1,000). Courtesy Elliot Magidson, MD.

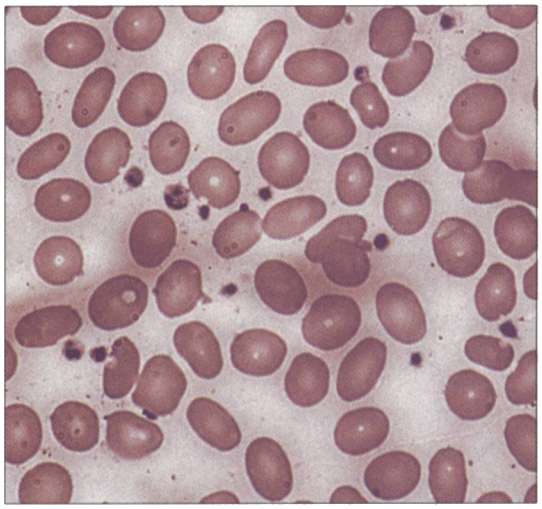

Fig 3. Peripheral blood smear from a patient with hereditary elliptocytosis. Moderate number of elliptocytes are shown (Wright stain, original magnification, ×1,000). Courtesy Elliot Magidson, MD.

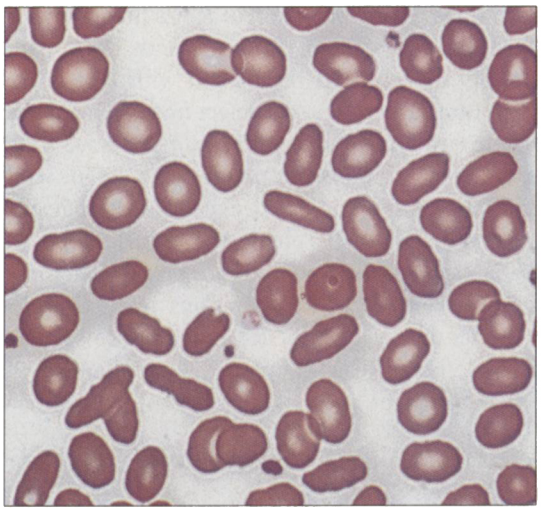

Fig 4. Peripheral blood smear from same patient as in Fig 3 after heating blood to 46°C. Slight increase in the number of elliptocytes but no increase in RBC fragmentation is shown (Wright stain, original magnification, ×1,000). Courtesy Elliot Magidson, MD.

Table 3. Laboratory Differentiation of RBC Membrane Disorders

Test	Result in Patients With: HS	HE	HPP	HST	HX
RBC morphology	Spherocyte	Elliptocyte	Spherocyte, elliptocyte, poikilocyte	Stomatocyte	Target cell
MCV	↑,↓, or normal	Slightly ↑, or normal	↓	↑	↑, or normal
MCHC	↑	Normal	↑	↓	↑
Osmotic fragility	↑	Normal	↑	↑	↓
Thermal sensitivity	NA	↑, fragments at 47–48°C	↑, fragments at 46°C	NA	NA

HS represents hereditary spherocytosis; HE, hereditary elliptocytosis; HPP, hereditary pyropoikilocytosis; HST, hereditary stomatocytosis; HX, hereditary xerocytosis; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; NA, not applicable.

associations between spectrin heterodimers that form spectrin tetramers and anomalous interactions between the junctional complexes at the distal ends of spectrin tetramers.[1] The resulting loss in skeletal integrity causes RBCs to be fragmented and misshapen.[3]

The principal defect in HE involves the horizontal membrane protein interactions, which are associated with several factors: defective spectrin chains,[4] defects or deficiencies in band 4.1,[5] glycophorin C deficiency, and the presence of an abnormal anion transport protein (band 3).[6] These atypical molecules cause RBCs to become elliptical in shape and to fragment under the stress of circulation.

HE is most commonly transmitted autosomally as a dominant allele. The HE condition, which may occur as a homozygous mutation or compound heterozygote with two mutations, produces variable degrees of clinical severity in affected patients.[1]

In HPP, RBCs have two abnormalities: an α-spectrin deficiency, resulting in defective vertical interactions, and a mutant spectrin that causes atypical horizontal interactions.[3] The combination leads to the formation of RBCs that resemble those of burn victims. Unlike HE mutations, HPP defects are recessive; the patient with HPP must therefore have inherited a defect from each parent. The parent with the spectrin trait will have mild HE or be asymptomatic, whereas the parent with the trait for spectrin deficiency is hematologically normal.[1] The HPP phenotype is also seen in patients who are homozygous or doubly heterozygous for one or two HE mutations, respectively.[1,2]

After the defect in the RBC membrane is determined, therapy can be initiated. For patients with HPP, treatment includes splenectomy, which may not alleviate the patient's symptoms, and transfusion if hemolysis is severe, chronic, or both.[7] With genetic testing, patients with these conditions can make informed decisions about having children. In the near future, gene therapy in the form of stem cell transplantation will become the treatment of choice.[8]

Role of the Laboratory

Because increased RBC hemolysis is seen in a variety of hereditary RBC membrane defects, the laboratory plays a key role in differentiating HPP from other hereditary defects such as spherocytosis, common HE, stomatocytosis and xerocytosis. The laboratory features of RBC membrane disorders are shown in Table 3. In HPP, morphologically abnormal RBCs are present,[1,7] and the MCV is usually decreased, depending on the number of RBC fragments present. An autohemolysis test shows increased RBC hemolysis that is not corrected by adding glucose.[7] In addition to showing abnormal heat sensitivity and fragmentation at 46°C, RBCs from patients with HPP demonstrate increased osmotic fragility both initially and after a 24-hour incubation.[8]

The anomaly most consistent with common HE is prominent elliptocytosis, in which atypical cells usually comprise greater than 25% of the RBCs. In uncomplicated cases of HE, hemoglobin levels usually exceed 12 g/dL (120 g/L) and reticulocyte counts, at 4%, are only slightly elevated. Tests for osmotic fragility (incubated and nonincubated) and autohemolysis show normal results. RBCs are mildly heat sensitive, and they become echinocytic and fragment at 47° to 48°C.[2]

Today, many specialty research laboratories use sophisticated techniques to accurately identify RBC protein deficiencies and genetic mutations associated with RBC membrane disorders.

Conclusion

The case study described calls attention to the clinical and laboratory features of HE and HPP, and shows how laboratory test results help to differentiate the disorders. To ensure correct diagnosis and prompt initiation of therapy, technologists must report changes in RBC morphology as accurately as possible. The patient benefits in the assurance that his or her disorder is being treated and monitored in the most appropriate manner.

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Acknowledgment

The authors thank the staff at Wichita Clinic for bringing this patient to their attention.

COMING UP IN FEBRUARY

Feature

HIV Viral Load Testing

Viral quantitation has replaced the CD4 count as the standard for monitoring therapy options and the progression of HIV. Increasingly sensitive procedures can detect wide ranges in the quantity of HIV nucleic acids. While patient care has improved because of these tests, they are technically involved and can take some time to master. Learn how laboratories are incorporating viral load testing into their work flow and what items should be considered when choosing a viral load assay for your laboratory.

CE Update

Transfusion Medicine I

Same-day surgeries have increased dramatically with managed care and so has the pressure on transfusion services. How can transfusion services best obtain specimens for type and screen in these conditions? Several institutions have established procedures that adhere to both regulatory and institutional policies as laboratorians continue to provide patients with safe blood products.

Scientific Communication

Neutrophils

Whether you call them polymorphonucleocytes, PMNs, or neutrophils, the physiology of the immune response they elicit is a fascinating topic. We've been taught that their primary role is to thwart infectious disease by clobbering bacteria and other invaders. Ironically, the reactive oxidants produced during the immune response are believed to promote inflammatory-induced diseases as well as cancer. Don't miss out on learning more about this interesting paradox in the February issue.

Corrections

The following correction is in regard to an errant quotation and incorrect title that appeared in the December 1998 Feature "Raising the Bar on Medicare Compliance," *Lab Med.*1998;29:740-746.

"The significance of the SmithKline case was that Medicare alleged performing additional tests such as ferritin with every panel was unnecessary and constituted fraud," emphasizes Daryl Vogel, MD, PhD, a pathologist at Skagit Valley Laboratories (a division of Dynacare) in Mount Vernon, Wash.

In the October 1998 issue an error appeared on page 616 of "Advancements in Blood Collection Devices" (*Lab Med.* 1998;29:616–622). The sixth sentence below the subhead "Laser Systems" should read:

"The wound created by lasers is smaller than that created by a standard 24-gauge lancet and approximately 100 µL of blood can be obtained."

In that same article the proper order of references 7 through 9 follows:

7. Meites S, Saniel-Banrey K. Preservation, distribution and assay of glucose in blood, with special reference to the newborn. *Clin Chem.* 1979;25:531–534.
8. Guder WG, Narayanan S, Wisser H, et al. *Samples: From the Patient to the Laboratory.* Darmstadt, Germany: GIT VERLAG, 1996.
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