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CONTINUING MEDICAL EDUCATION
ΣΥΝΕΧΙΖΟΜΕΝΗ ΙΑΤΡΙΚΗ ΕΚΠΑΙΔΕΥΣΗ

Hematology Quiz – Case 67

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ΑΡΧΕΙΑ ΕΛΛΗΝΙΚΗΣ ΙΑΤΡΙΚΗΣ 2025, 42(1):141–144

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A 54-year-old woman was admitted to the surgery service of a tertiary hospital because of "splenomegaly – scheduled for therapeutic splenectomy". A hematology consultation was requested before the scheduled surgery. Splenomegaly was first noted at the age of 20 years during a routine examination; no further diagnosis was made and a "watch and wait" approach was initiated. Eight years before admission, she underwent cholecystectomy because of gallstones; then, she had a hematocrit level of 36.8%. Two years before admission, declining hematocrit values had been noted. She was referred to a hematologist who noted a large spleen descending 10 cm below costal margin and a diagnosis of "chronic hemolytic anemia" was made. She had had regular follow-up visits with her hematologist, and during the previous five months before her current presentation she had been transfused with two units of packed red cells. The record of her pre-transfusion hemoglobin values was not available. On admission to the surgical ward she had a hematocrit value of 30%, normal white-cell and platelet counts, mean corpuscular volume (MCV) 85 fL, mean corpuscular hemoglobin (MCH) 30.5 pg, mean corpuscular hemoglobin concentration (MCHC) 35.7 g/dL, reticulocytes 4.4%, lactate dehydrogenase (LDH) 345 U/L (normal: 125–220), total bilirubin 1.69 mg/dL (direct 0.4), ferritin 62.8 ng/mL (normal: 17–165), vitamin B12 443 pg/mL (normal: 170–590), folic acid 13 ng/mL (normal: 1.5–5.5), and a negative direct Coombs test. Representative images of the peripheral-blood smear are shown in figures 1–7. Figure 8 is the patient's bone-marrow aspirate smear stained with May-Grünwald-Giemsa, and figure 9 is the iron stain.

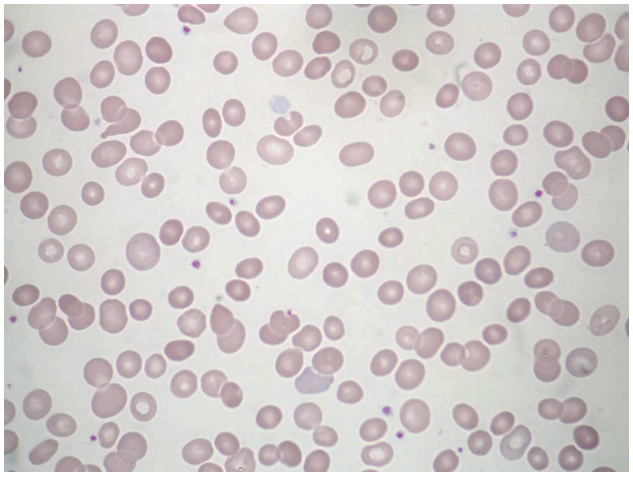


Figure 1.

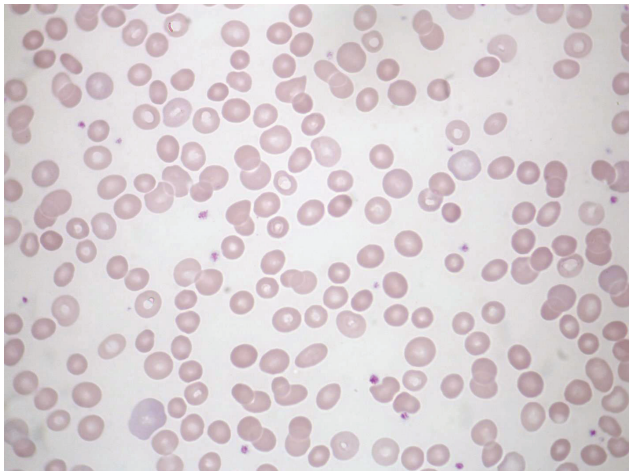


Figure 2.

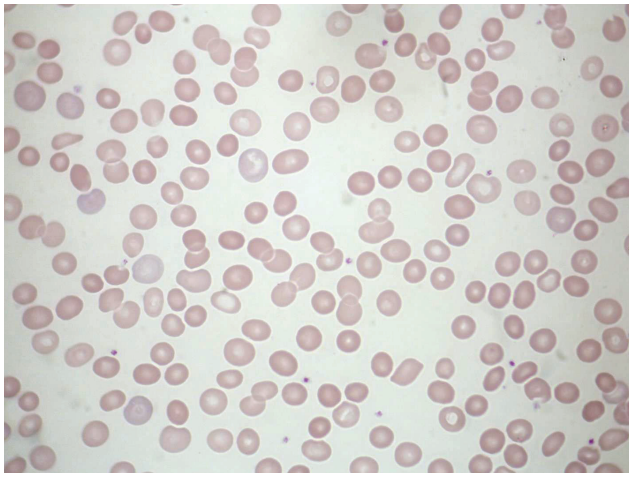


Figure 3.

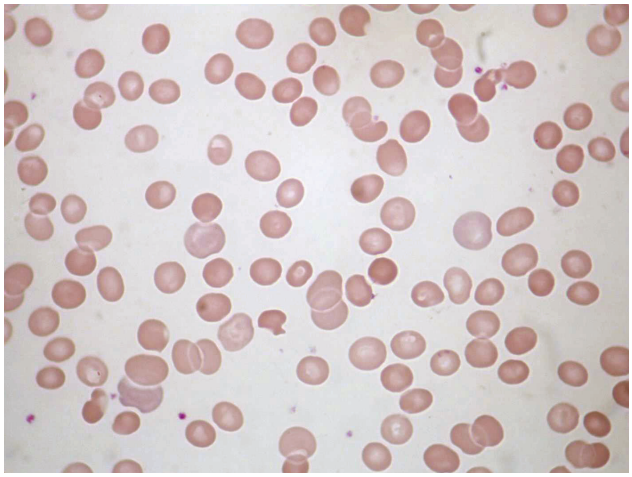


Figure 6

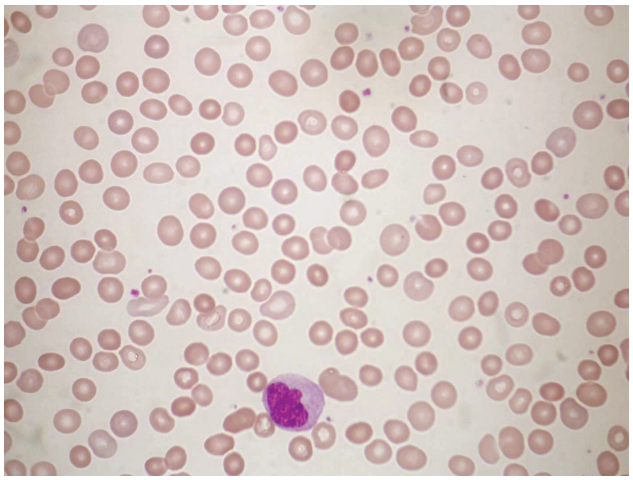


Figure 4

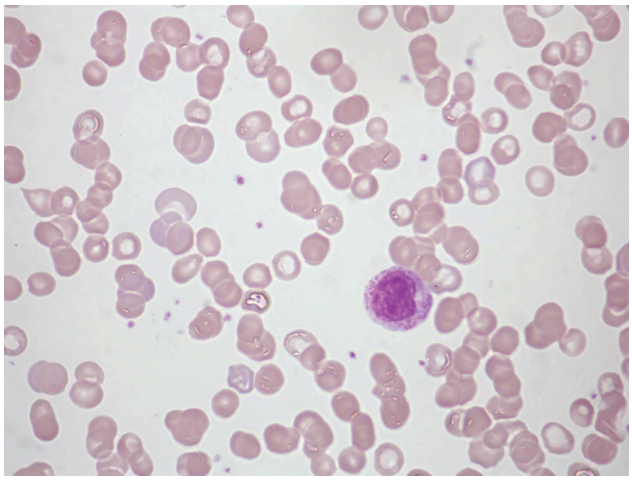


Figure 7

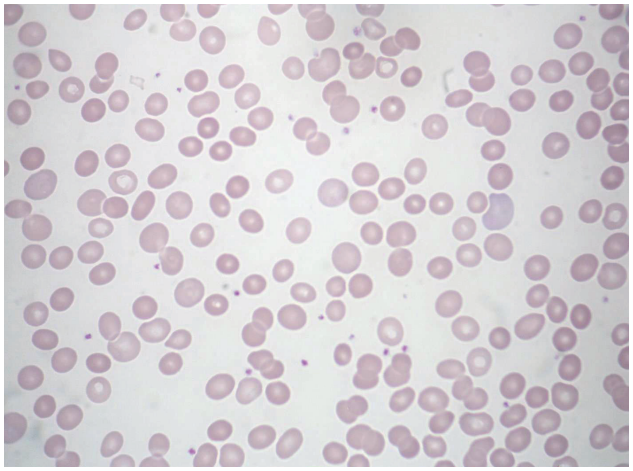


Figure 5

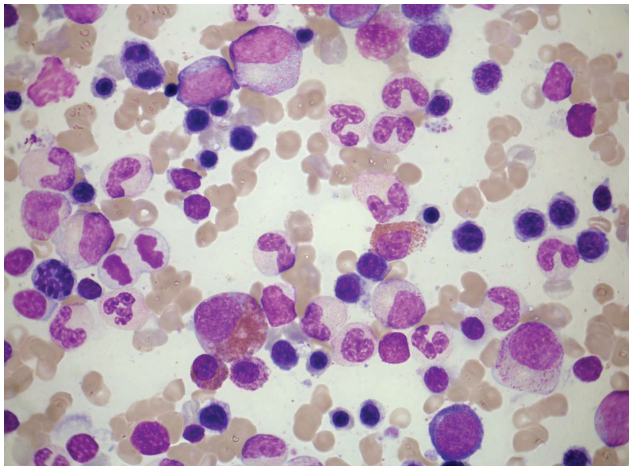


Figure 8

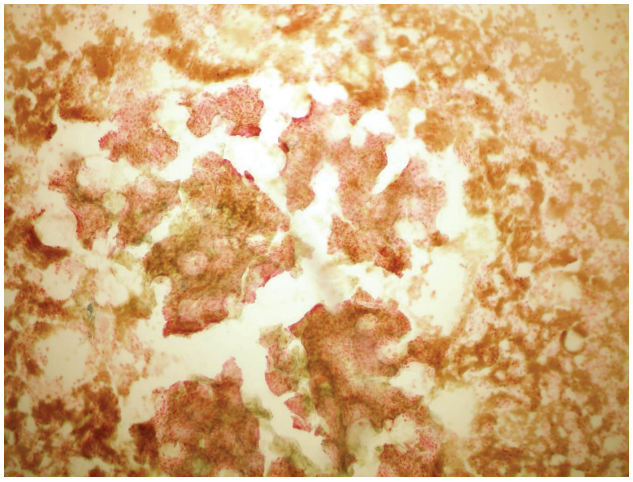
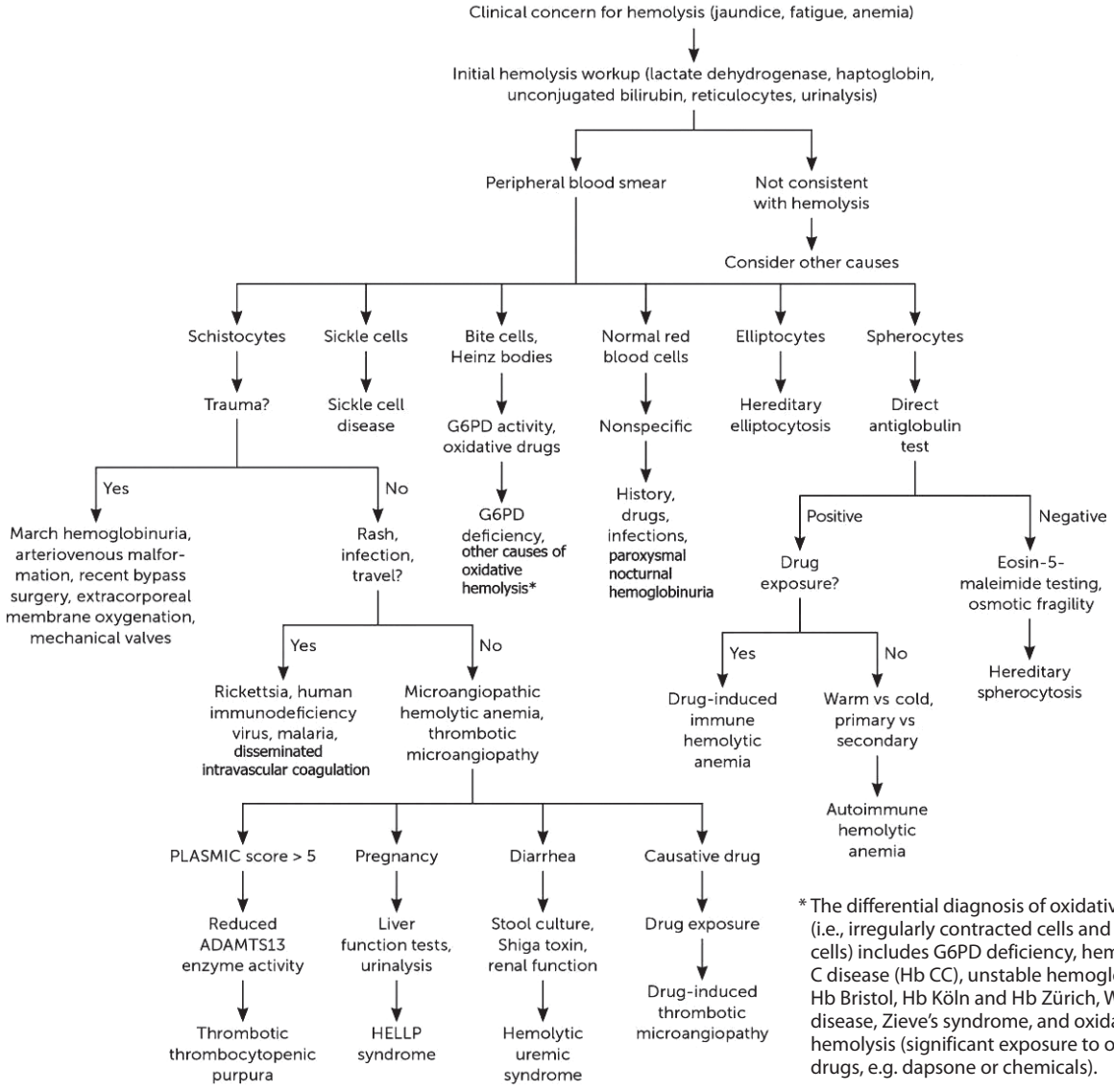


Figure 9

Comment

Once the laboratory diagnosis of hemolytic anemia is established, the next step is to carefully examine the morphologic features of the red cells in the peripheral-blood smear. The peripheral-blood smear (figures 1–7) shows spherocytosis and polychromatophilic red cells. Spherocytes are formed when the biconcave morphology of a red blood cell is lost, its size is reduced and eventually becomes spherical whilst losing or reducing its central pallor. In this patient, red-cell morphology is consistent with spherocytes that retain some of their central pallor. This finding is typical of hereditary spherocytosis (HS). There are also a few tear drop cells indicative of the patient's splenomegaly and rare mushroom-shaped red cells (pincer cells), as shown in figure 6.

Bone-marrow aspiration is not routinely performed in patients with hemolytic anemia. In this case, however, the presence of sple-



* The differential diagnosis of oxidative hemolysis (i.e., irregularly contracted cells and or blister cells) includes G6PD deficiency, hemoglobin C disease (Hb CC), unstable hemoglobins e.g. Hb Bristol, Hb Köln and Hb Zürich, Wilson's disease, Zieve's syndrome, and oxidant-induced hemolysis (significant exposure to oxidant drugs, e.g. dapsone or chemicals).

Figure 10. Morphologic approach to hemolytic anemia (modified from reference 1).

nomegaly along with myelocytes in the peripheral blood (fig. 7) prompted us to obtain an aspirate in order to rule out hematologic malignancy. The aspirate smear was cellular. Erythroid hyperplasia was noted, consistent with a hemolytic state, with no increase in blasts and no atypical cells (fig. 8). We saw evidence of reduced iron stores in fragments (fig. 9). This finding can be seen in chronic hemolytic anemias with adequate compensation, as a consequence of increased erythropoiesis.

The approach of a patient with hemolytic anemia begins with an evaluation of the peripheral-blood film (fig. 10). In this case, the presence of spherocytes raised suspicion for autoimmune hemolytic anemia but a Coombs test came back negative which makes the possibility of immune hemolytic anemia very unlikely. The next step for Coombs-negative spherocytic hemolytic anemia is the erythrocyte osmotic fragility test or the eosin-5-maleimide (EMA) binding assay (if available). In the osmotic fragility test, red blood cells are incubated with increasingly hypotonic NaCl solutions and the percentage of red-cell lysis is recorded. Normally, 50% of red blood cells are lysed in a solution with a concentration of 0.5% NaCl. The EMA binding test uses flow cytometry to determine the percentage of fluorochrome that binds to the red blood cells, reflecting EMA bound to transmembrane protein band 3. More specifically, red blood cells are incubated with EMA dye which covalently binds to band 3 and other proteins (Rh proteins, Rh glycoprotein, and CD47) on the red-cell surface. The mean fluorescence of EMA-stained red blood cells in patients with HS is lower as compared with normal red cells due to the decreased amount of band 3 protein. Reduced values are indicative of HS, but they may also occur in other situations such as hereditary pyropoikilocytosis and type II congenital dyserythropoietic anemia (HEMPAS).

A diagnosis of HS was made on the basis of the clinical history and peripheral blood findings. HS is a group of diseases caused by mutations in the genes of ankyrin (ANK1), β-spectrin (SPTB), band 3 (SLC4A1/EPB3), α-spectrin (SPTA), or protein 4.2 (EPB42) (in decreasing order of incidence). They are generally inherited as autosomal dominant conditions, but autosomal recessive and new-onset sporadic mutations have also been reported. These mutations lead to the destabilization of the cytoskeleton and eventually loss of the lipid bilayer. The loss of lipids is responsible for the characteristic spherocytic morphology in HS.

Not all patients with HS are diagnosed at childhood. Occasionally, the diagnosis is made during adulthood. Family history is negative in approximately 25% of cases. There is large phenotypic variation and disease severity may differ significantly. Findings from the patient's history that help us include the presence of jaundice, splenomegaly, and brown faceted gallstones due to the hyperbilirubinemia of hemolysis (pigmented gallstones composed of bilirubin polymers). The most outstanding findings in the full blood count are in the erythrocyte indices. MCV –even though we are facing a hemolytic anemia– can be reduced due to loss of membrane and or iron deficiency owing to chronic hemolysis and the increased consumption of hematinic factors. The compensation in chronic hemolytic anemia may be less pronounced than that in acute hemolytic anemia with less reticulocytes, but may be enough to keep the hematocrit in acceptable values but not enough to make it normal. In HS, the MCHC is frequently increased because there is no defect in hemoglobin production, but a decrease in the cell volume due to shrinkage of the cell membrane, which in turn increases the percentage of hemoglobin per cell.

The decision of the consulting hematologist was to proceed with the splenectomy. The patient had an uneventful recovery and she was given instructions to continue her follow-up in the outpatient clinic. After splenectomy, the hemoglobin was maintained >11 g/dL, with no need for transfusions.

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