A rare case of Diamond Blackfan anemia: identifying the causative mutation using NGS

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CAP TODAY and the Association for Molecular Pathology have teamed up to bring molecular case reports to CAP TODAY readers. AMP members write the reports using clinical cases from their own practices that show molecular testing's important role in diagnosis, prognosis, and treatment. The following report comes from Columbia University Medical Center in New York. If you would like to submit a case report, please send an email to the AMP at amp@amp.org. For more information about the AMP and all previously published case reports, visit www.amp.org.

July 2016—Diamond Blackfan anemia is a rare, inherited bone marrow failure syndrome manifesting as marked red cell aplasia and variable congenital anomalies. We report here a case of Diamond Blackfan anemia, which underscores the role of an integrated diagnostic workflow including hematopathologic evaluation and next-generation sequencing for establishing the diagnosis and potential management of rare, inherited bone marrow failure syndromes.

Case presentation and history. A 42-day-old female infant who was born at 37 weeks gestation presented at an outside medical center for evaluation of anemia. Prenatal history was notable for paternal HIV (mother and infant both tested negative), maternal urinary tract infection, and preterm labor. There was no family history of anemia; the patient had two healthy maternal half siblings and three healthy paternal half siblings.

In the newborn period, the infant was feeding well without respiratory distress. On day two of life, marked pallor and a 2/6 systolic ejection murmur was noted. Subsequent lab evaluation revealed low hemoglobin (8 g/dL) and a reticulocyte index of 3.4 percent. All other hematologic parameters and bilirubin levels were normal, prompting a hematology consultation. A peripheral smear showed the presence of all white blood cell lineages with normal morphology; platelets were increased and displayed variable size (small to large). Red blood cells were decreased and schistocytes, elliptocytes, spherocytes, and Heinz bodies were not present. Hemoglobin on day three remained stable with an appropriate reticulocyte count and no evidence of jaundice.

As the infant had no signs of hemolysis, and the clinical picture was consistent with a compensated anemia, the presence of infectious etiologies or structural abnormalities was evaluated. She had negative studies for HIV, parvovirus, toxoplasmosis, and cytomegalovirus infection. She had a normal electrocardiogram and echocardiogram except that mild mitral and tricuspid valve regurgitation were noted. Hemoglobin analysis by high-performance liquid chromatography showed no abnormalities (HbF, 45 percent; HbA, 53.6 percent; and HbA2, 1.3 percent). This raised the suspicion for a congenital anemia. On day 21 of life, a repeat CBC showed an Hb of 4.7 g/dL, prompting admission for repeat evaluation and possible packed red blood cell (PRBC) transfusion. The infant continued to feed well and showed no signs of cardiovascular compromise. She was transfused PRBCs, and the post-transfusion hemoglobin was approximately 7 g/dL. Despite appearing well clinically, her hemoglobin and reticulocyte counts continued to decline. All other cell lineages remained normal and the platelet count ranged from normal to high. On day 42 of life, she had a hemoglobin of 5.5 g/dL with reticulocyte index of 1.8 percent. At this time, she was referred to our institution for further hematology diagnostic workup and continuation of care.

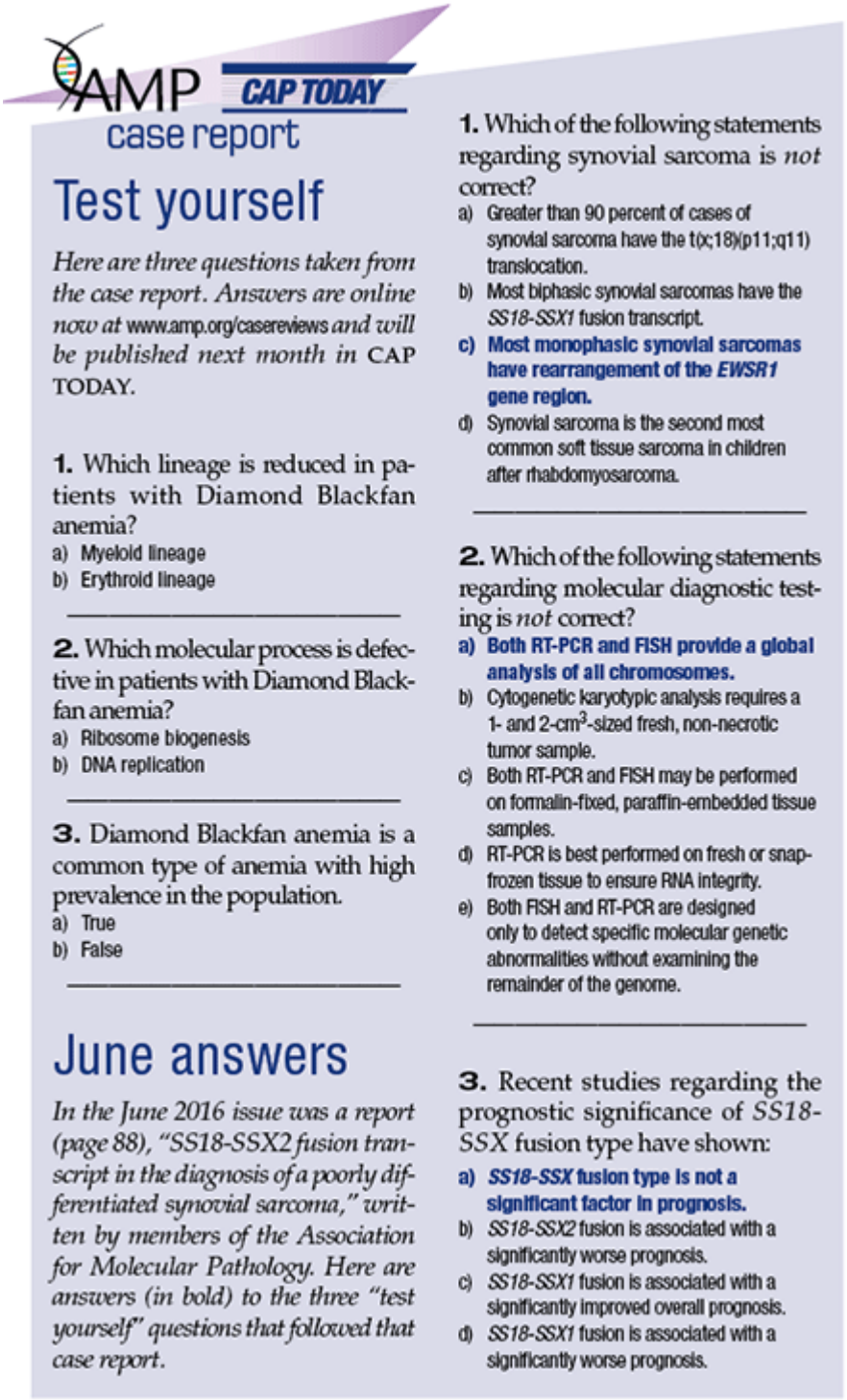
Results. Physical examination revealed that our patient was non-dysmorphic and normal appearing with no evident anomalies. The feeding and sleeping patterns were normal, and the infant remained asymptomatic despite her normocytic anemia. Since the patient had no compensatory reticulocytosis, a congenital anemia associated

with bone marrow failure, such as Diamond Blackfan anemia, was suspected.

Differential count of peripheral blood on day 50 of life showed the following abnormal parameters: RBC, 1.21 × 1012/L (normal range 3.00–5.40 × 1012/L); PLT, 936 × 109/L (range 165–415 × 109/L); Hb, 4.1 g/dL (range 10.0–18.0 g/dL); Hct, 12.3 percent (range 31.0–55.0 percent); MCH, 33.9 pg (range 26.7–31.9 pg); RDW, 21.6 percent (range ≤14.4 percent); and reticulocyte index, 0.5 percent. Morphologic review of the bone marrow aspirate exhibited erythroid hypoplasia, megakaryocytic hyperplasia, and eosinophilia (Fig. 1A and B); platelets were markedly increased. The myeloid to erythroid ratio was >>10:1. Cells of the erythroid series were rare, exhibiting an apparent left shift and megaloblastoid features, findings that are consistent with severe erythroid hypoplasia. Lymphocytes were present in appropriate quantities for age, including a significant population of hematogones. The pattern of iron incorporation in the smears was difficult to assess due to the paucity of erythroid precursors, but ringed sideroblasts were not observed. Erythrocyte adenosine deaminase (ADA) levels were elevated (1143 mU/g Hb, reference units: 400–900).

Flow cytometric analysis of the bone marrow aspirate highlighted a predominant population of T cells without significant downregulation or loss of the pan T-cell antigens and a small population of polytypic B cells, many expressing CD5, in the CD45 bright, low side scatter gate. On analysis of the CD45-dim, low-intermediate side scatter (blast) gate, a predominant population of hematogones was detected. No elevation in CD34+, CD117+ myeloblasts was noted. There was no evidence of non-Hodgkin lymphoma or acute leukemia.

Cytogenetic analysis of the bone marrow aspirate to rule out primary lymphohematopoietic neoplasm was carried out. A normal 46,XX female karyotype was found in all of the 20 cells examined. FISH analysis using a myelodysplastic syndrome panel of chromosome probes (5q, 7q, 8, 20q) showed no evidence of clonal abnormalities.



AMP CAP TODAY
case report

Test yourself

Here are three questions taken from the case report. Answers are online now at www.amp.org/casereviews and will be published next month in CAP TODAY.

1. Which lineage is reduced in patients with Diamond Blackfan anemia?
a) Myeloid lineage
b) Erythroid lineage

2. Which molecular process is defective in patients with Diamond Blackfan anemia?
a) Ribosome biogenesis
b) DNA replication

3. Diamond Blackfan anemia is a common type of anemia with high prevalence in the population.
a) True
b) False

June answers

In the June 2016 issue was a report (page 88), "SS18-SSX2 fusion transcript in the diagnosis of a poorly differentiated synovial sarcoma," written by members of the Association for Molecular Pathology. Here are answers (in bold) to the three "test yourself" questions that followed that case report.

1. Which of the following statements regarding synovial sarcoma is *not* correct?
a) Greater than 90 percent of cases of synovial sarcoma have the t(x;18)(p11;q11) translocation.
b) Most biphasic synovial sarcomas have the *SS18-SSX1* fusion transcript.
c) Most monophasic synovial sarcomas have rearrangement of the *EWSR1* gene region.
d) Synovial sarcoma is the second most common soft tissue sarcoma in children after rhabdomyosarcoma.

2. Which of the following statements regarding molecular diagnostic testing is *not* correct?
a) Both RT-PCR and FISH provide a global analysis of all chromosomes.
b) Cytogenetic karyotypic analysis requires a 1- and 2-cm^3-sized fresh, non-necrotic tumor sample.
c) Both RT-PCR and FISH may be performed on formalin-fixed, paraffin-embedded tissue samples.
d) RT-PCR is best performed on fresh or snap-frozen tissue to ensure RNA integrity.
e) Both FISH and RT-PCR are designed only to detect specific molecular genetic abnormalities without examining the remainder of the genome.

3. Recent studies regarding the prognostic significance of *SS18-SSX* fusion type have shown:
a) *SS18-SSX* fusion type is not a significant factor in prognosis.
b) *SS18-SSX2* fusion is associated with a significantly worse prognosis.
c) *SS18-SSX1* fusion is associated with a significantly improved overall prognosis.
d) *SS18-SSX1* fusion is associated with a significantly worse prognosis.

The patient's skeletal survey and renal sonogram were unremarkable; abdominal ultrasound ruled out hepatosplenomegaly. Based on the presence of anemia without reticulocytosis, erythroid hypoplasia on bone marrow examination (all other cell lineages being normal), and elevated erythrocyte ADA level, a clinical diagnosis of Diamond Blackfan anemia was suggested.

Whole exome sequencing of the peripheral blood sample identified a pathogenic, heterozygous, initiation codon variant predicted to result in an untranslated protein in RPS26 (c.1A>G, p.Met1?, previously p.Met1Val; OMIM:603701; Fig. 2), a ribosomal protein gene known to cause autosomal dominant Diamond Blackfan anemia 10 (OMIM:613309). The mutation seen in the patient has been previously reported.¹ Nucleotide position 1A-G transition in exon 1 of the RPS26 gene causes a met1-to-val (M1V) substitution that eliminates the start codon and is predicted to result in loss of translation of the ribosomal protein.

Discussion. Diamond Blackfan anemia, or DBA, is a rare, congenital, hypoplastic anemia with an estimated incidence ranging from 1:100,000 to 1:200,000 live births.2 It usually presents in the first year of life. Unlike other aplastic anemias, such as Fanconi anemia, the defect is isolated to the red blood cell lineage. Clinically, the anemia manifests as a macrocytic anemia with elevated mean corpuscular volumes, erythrocyte ADA, HbF, and a decreased reticulocyte count.2 The bone marrow shows a near absence of erythroid precursors and unremarkable megakaryocytic and neutrophilic lineages. In about 30 to 50 percent of DBA cases, growth retardation and congenital malformations affecting craniofacial structures, upper limb, heart, and urinary systems can be seen. There is also an increased risk of malignancy. The disorder is thought to result from increased sensitivity of erythroid progenitors to apoptosis, leading to erythropoietic failure.2 Currently, DBA is managed with transfusions, corticosteroid therapy, or stem cell transplantation.

Mutations in 15 ribosomal genes are known to cause DBA. In most cases, it is inherited in an autosomal dominant manner except two ribosomal genes localized on the X chromosome, mutations in which are inherited in an X-linked recessive manner. Point mutations and large deletions in the ribosomal genes have been described as disease-causing mutations. Both incomplete penetrance and variable expressivity have been reported for DBA.2 Congenital anomalies and growth retardation, which may be seen in DBA, were not observed in our patient.

Measurements at age 21 months showed her to be in the 91st percentile for height and 96th percentile for weight. Individuals with the RPS26 gene p.Met1? mutation have been reported to show variable response to steroid therapy.1 Our patient was initially managed with transfusion therapy, and at about age one she was put on a steroid trial that failed and chronic transfusions were therefore resumed. At the time of this writing, she is 22 months old and maintained on PRBC transfusions. Her care has been complicated by chronic transfusion induced iron overload and hospital admissions due to possible infections. She is a candidate for bone marrow transplantation.

Methods. As part of the diagnostic workup, morphologic evaluation of the peripheral blood smear and bone marrow aspirate, flow cytometry, and cytogenetic analysis were performed. Because targeted testing for the 13 known genes associated with DBA identifies mutations in 55 percent of cases of DBA, and due to insurance coverage and cost issues related to send-out versus in-house testing, whole exome sequencing was performed on DNA isolated from the peripheral blood sample of the patient. WES testing was offered to the parents but they declined.

1. Doherty L, Sheen MR, Vlachos A, et al. Ribosomal protein genes RPS10 and RPS26 are commonly mutated in Diamond-Blackfan anemia. *Am J Hum Genet.* 2010;86(2):222–228. Erratum in: Am J Hum Genet. 2010;86(4):655.
2. Vlachos A, Ball S, Dahl N, et al; Participants of Sixth Annual Daniella Maria Arturi International Consensus Conference. Diagnosing and treating Diamond Blackfan anaemia: results of an international clinical consensus conference. *Br J Haematol.* 2008;142(6):859–876.

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