Clinical Case Study

A Young Adult with Aplastic Anemia and Gray Hair

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CASE DESCRIPTION

A man in his early twenties presented with hematuria and moderate pancytopenia [white blood cell count, 1.9×10^9/L (reference, 4.2×10^9/L to 9.9×10^9/L); hemoglobin, 13.3 g/dL (reference, 13.0–17.4 g/dL); platelets, 61×10^9/L (reference, 140×10^9/L to 440×10^9/L)]. The physical examination was unremarkable, as were routine laboratory tests. A bone marrow (BM)[6] aspirate and biopsy demonstrated hypocellularity (20%) without dysplasia. Results for BM cytogenetics, fluorescence in situ hybridization (FISH), and a diexpoybutane test for Fanconi anemia were normal. Flow cytometry findings for peripheral blood showed no lymphoproliferative or myeloid-maturation disorder, nor any protein defect consistent with paroxysmal nocturnal hemoglobinuria. Over the next 5 years, the patient remained asymptomatic and transfusion free with stable blood counts and an unchanged BM histology.

As part of a review of potential therapeutic interventions, he sought a second opinion, at which time the patient recounted a history of recurrent urethral strictures from ages 6 to 9 years and again at age 22 years. His hair had started to gray at 11 years, and his hairline to recede by age 16. There was a family history of similar findings. He described his fingernails and toenails as always "embarrassingly" dry and cracked. He recounted having had excess tearing and that friends would note he was crying although he was unaware of tear production. On examination, the patient had hair thinning, graying, and mild frontal balding. He had subtle, nonblanching, slightly reddish brown reticular pigmentation over his upper anterior and

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QUESTIONS TO CONSIDER

1. What are the causes of aplastic anemia (AA)?
2. Given this patient's symptoms, what might be the diagnosis?
3. What laboratory test would be useful for the diagnosis?

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posterior thorax. All of his nails were markedly dystrophic. There was a flat 1×1.5 cm white lesion on the upper hard palate. The remainder of his examination was unremarkable. His blood counts had been quite stable over the 5 antecedent years: white blood cell count, 1.4×10^9/L to 3.8×10^9/L (reference, 4.2×10^9/L to 9.9×10^9/L); absolute neutrophil count, 0.71×10^9/L to 2.7×10^9/L (reference, 2.4×10^9/L to 7.6×10^9/L); hemoglobin, 12.9–14.5 g/dL (reference, 13.0–17.4 g/dL), with marked macrocytosis (mean corpuscular volume, 105–111 fL; reference, 82.0–100 fL); and platelets, 46×10^9/L to 71×10^9/L (reference, 140×10^9/L to 440×10^9/L).

DISCUSSION

The patient presented as an adult with moderate AA, which is defined by a hypocellular BM with decreased peripheral blood counts in >1 cell lineage. Most cases of AA are classified as idiopathic or acquired and are most commonly thought to be due to immune dysregulation, perhaps owing to viral infection. Exposures to a substantial radiation dose, certain drugs, and chemicals, as well as the occurrence of some autoimmune syndromes, have also been associated with marrow failure. Hematologic conditions, including myelodysplasia, paroxysmal nocturnal hemoglobinuria, and, rarely, leukemia can also present as AA. Alternatively, careful evaluation of the patient's history, physical examination, and family history may suggest an inherited BM failure syndrome (IBMFS) as the etiology. This patient demonstrated several features of dyskeratosis congenita (DC) in its classic form (nail dystrophy, mucosal leukoplakia, and skin pigmentation changes) and in concert with other abnormalities, including BM failure, urethral strictures, excessive tear production (epi-
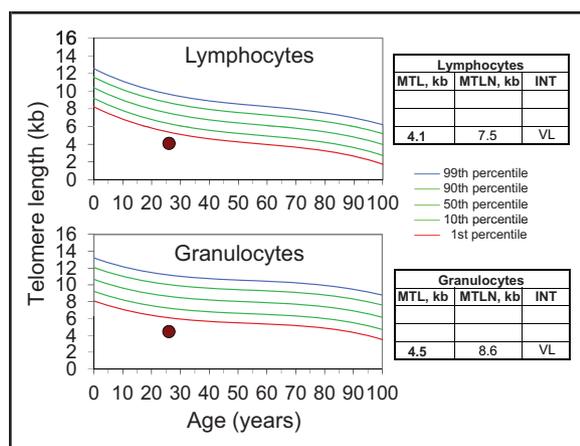
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Fig. 1. Measurement of MTL by flow-FISH in lymphocytes and granulocytes, plotted as the percentile of age-adjusted normal values.

The patient shows a very low MTL (<1st percentile) in both blood cell types. MTLN, normal MTL at a given age (50th percentile); INT, TL interpretation; VL, very low (<1st percentile).

phora), premature balding and gray hair, and pulmonary disease *(1)*.

PATIENT FOLLOW-UP

On the basis of a presumptive clinical diagnosis of DC, we undertook measurements of telomere length (TL), which showed both his lymphocytes and granulocytes had a markedly reduced (<1st percentile) median TL (MTL) [4.1 kb vs 7.5 kb (50th percentile for age) and 4.5 kb vs 8.6 kb (50th percentile for age), respectively], thereby confirming the diagnosis (Fig. 1). Analyses for mutations in genes known to be associated with DC included sequencing of *DKC1*[7] (dyskeratosis congenita 1, dyskerin), *TINF2* [TERF1 (TRF1)-interacting nuclear factor 2] exon 6, and *TERC* (telomerase RNA component). Deletion/duplication testing of the *TERC* gene was also undertaken. No abnormalities were identified. Bone densitometry (dual-energy x-ray absorptiometry) results were normal. Pulmonary-function testing revealed a carbon monoxide–diffusing capacity of the lung (or DLCO) value of 17.0 mL · mmHg^{-1} · min^{-1} (45% of predicted). Biopsy of

the hard-palate lesion showed mild epithelial dysplasia and hyperkeratosis.

The identification of genetic mutations underlying IBMFSs over the past 20 years has revealed the marked variation in clinical onset and manifestations caused by similar mutations. Thus, both pediatric and adult hematologists must maintain a high level of suspicion for genetic causes when evaluating patients with BM failure. Multiple inheritance patterns (autosomal dominant and recessive, X-linked, sporadic), variable penetrance, pleiotropy, and genetic anticipation complicate the diagnosis of DC *(2)*. Patients with DC-related disorders may present along a clinical spectrum encompassing severe multisystem disease in infancy (e.g., Hoyeraal–Hreidarsson and Revesz syndromes) and later-onset, tissue-restricted disorders such as pulmonary fibrosis. Mutations in one of 8 genes involved in telomere biology—*DKC1*, *TINF2*, *TERC*, *TERT* (telomerase reverse transcriptase), *NHP2* [NHP2 ribonucleoprotein homolog (yeast)], *NOP10* [NOP10 ribonucleoprotein homolog (yeast)], *WRAP53* (WD repeat containing, antisense to TP53; formerly *TCAB1*), and *CTC1* (CTS telomere maintenance complex component 1)—can be identified in approximately 50% of patients with clinical features of classic DC, the remainder being as yet uncharacterized *(3, 4)*. Telomeres occur at the ends of chromosomes and consist of hundreds to thousands of hexanucleotide repeats [(TTAGGG)$_n$], which undergo attrition with each cell division. Multiprotein complexes (e.g., including components encoded by *TINF2* and *CTC1*) maintain telomeres, and the ribonucleoprotein telomerase [composed of the products of the *TERC*, *TERT*, *DKC1*, *TCAB1* (i.e., *WRAP53*), *NHP2*, and *NOP10* genes] replaces hexanucleotide repeats by reverse transcription to counteract telomere attrition. The genetic lesions in DC compromise telomere integrity, impairing the self-renewal and regenerative capacity in cells, and presumably explain the premature failure of multiple organs in affected individuals *(5)*.

Defective telomere maintenance is a common feature across DC genotypes that can be exploited for diagnosis. Lansdorp and colleagues have developed a CLIA-certified flow cytometry–based FISH (flow-FISH) approach for quantifying TL in peripheral blood cells *(6)*. Fluorescently labeled peptide nucleic acid probes (CCCTAA)$_3$ are hybridized to telomeres in permeabilized peripheral blood cells. The number of probes bound to telomeres is proportional to TL, and the total fluorescence intensity of probes bound to all 92 telomere ends of the 46 chromosomes is read out as the MTL per cell. Because telomeres normally shorten

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[7] Human genes: *DKC1*, dyskeratosis congenita 1, dyskerin; *TINF2*, TERF1 (TRF1)-interacting nuclear factor 2; *TERC*, telomerase RNA component; *TERT*, telomerase reverse transcriptase; *NHP2*, NHP2 ribonucleoprotein homolog (yeast); *NOP10*, NOP10 ribonucleoprotein homolog (yeast); *WRAP53* (formerly *TCAB1*), WD repeat containing, antisense to TP53; *CTC1*, CTS telomere maintenance complex component 1.

in blood cells with age, Lansdorp et al. established age-adjusted norms for MTL as measured by flow-FISH analysis. Simultaneous antibody staining for cell surface markers allows measurement of the MTL in specific subsets of peripheral blood cells (e.g., lymphocytes, granulocytes). Comparing the patient's sample with healthy controls allows a percentile score normalized for age and cell type to be assigned to the patient's MTL.

Alter et al. used flow-FISH to measure the MTL in subsets of peripheral blood cells from 65 DC patients (defined as those with classic clinical abnormalities or with a known pathogenic mutation) vs 127 clinically healthy family members *(7)*. Using MTL scores of <1st percentile ("very low") as a cutoff, Alter et al. showed that flow-FISH provides a sensitivity and a specificity for diagnosing DC of 97% and 91%, respectively, with total lymphocytes, and 97% and 82% with granulocytes. Sensitivity and specificity for diagnosing DC with lymphocyte subsets were investigated, but the best performance characteristics of flow-FISH for a single lineage were obtained from measuring MTL in total lymphocytes. These important studies validated flow-FISH as a test with diagnostic utility for DC, and we used this test to support the clinical diagnosis of DC in this patient (Fig. 1).

Definitive genetic diagnosis of DC remains limited by the need to sequence a large, yet incomplete set of genes and by the difficulty in establishing the pathogenicity of variant sequences. *DKC1* mutations are found in 25%–30% of DC cases. *DKC1* is X-linked, and males are affected, whereas female carriers are generally clinically silent. *TINF2* mutations account for approximately 15% of DC cases, are autosomal dominant, and are frequently sporadic. *TERC* and *TERT* mutations (5%–10% each of all cases) are found in autosomal dominant and recessive DC and show disease anticipation, such that successive generations not only inherit the genetic lesion but also have shorter telomeres, which may produce an earlier onset of clinical manifestations *(8)*. Somatic reversion of the *TERC* gene, likely driven by a resulting selective growth advantage in hematopoietic stem cells, has been observed in DC and is a potential confounder of genetic testing *(9)*. With sufficient clinical concern, a normal result in a *TERC* analysis of peripheral blood DNA should be followed by testing of other somatic cells. Our patient's history was insufficient to determine the inheritance pattern, and a combination of CLIA-certified and laboratory-based testing ruled out mutations in *DKC1*, *TINF2*, *TERC*, and *TERT*. Analysis of the parents' and sibling's MTLs by flow-FISH might clarify the inheritance pattern, and additional candidate gene and exome sequencing might determine the genetic basis of the patient's DC.

Establishing the diagnosis of IBMFS vs acquired AA has significant therapeutic implications. As in this case, delayed and mild presentations must be considered in adults with BM failure. The BM failure of IBMFS generally responds poorly to the cyclosporine A and antithymocyte globulin immunosuppressive regimen used for acquired AA in the absence of a matched sibling donor for hematopoietic cell transplantation (HCT). Moreover, HCT preparative regimens used for AA are associated with unacceptable organ toxicity in DC patients, whereas newer, disease-specific preparative regimens of reduced intensity have yielded improved outcomes. When any IBMFS is suspected, it is imperative to rule out subclinical disease in potential family donors for HCT. After BM failure, the leading causes of death in DC include head, neck, and anogenital squamous cell cancers, pulmonary fibrosis, and cirrhosis *(10)*. Although the pathophysiological relationship of defective telomere integrity to these complications remains speculative, the range of potentially fatal manifestations emphasizes the relevance of family screening, even absent HCT considerations. The diagnosis of DC and other IBMFSs therefore not only has an impact on immediate therapy but also mandates coordinated multidisciplinary care, long-term surveillance, anticipatory guidance, and family and genetic counseling.

POINTS TO REMEMBER

- Aplastic anemia can occur as a consequence of immune dysregulation and toxic exposures, and as a manifestation of several hematologic disorders, including myelodysplasia and IBMFS.

- A genetic cause should be considered in patients of all ages who present with AA. A basic evaluation for DC should include a careful physical examination, medical history, and family history.

- Genetic testing that should be considered in evaluating DC includes measurement of TL in peripheral blood cells and genetic-mutation analysis of candidate genes.

- Although DC is caused by lesions in several genes known to compromise telomere maintenance, the responsible gene(s) remain unknown in 50% of cases.

- Establishing a diagnosis of DC vs idiopathic AA has major implications for the patient and the family.

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Commentary

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The authors present a classic case of dyskeratosis congenita (DC) and nicely summarize the variability in clinical and diagnostic findings in patients with inherited bone marrow failure syndromes (IBMFSs). Owing to end-organ dysfunction, an increased risk for various cancers, and the need for appropriate genetic counseling, accurate diagnosis is essential. Although this patient demonstrates the classic findings of DC, many patients present with more subtle findings, making the distinction between IBMFS subtypes and acquired bone marrow failure difficult, further highlighting the necessity to combine clinical and diagnostic modalities for proper diagnosis.

There is tremendous heterogeneity in the clinical presentation of IBMFSs, likely because of comparable diversity in genetic causation and penetrance across the various subtypes. As this case demonstrates, a genetic cause is found in only about 50% of individuals with DC. Because the genetic underpinnings of DC remain incompletely characterized, variants in genes associated with telomere maintenance are often assumed to be causative, when, in some cases, they may be of ethnic or private origin and have little effect on telomere biology when analyzed functionally, potentially leading to misdiagnosis *(1)*.

Telomere length analysis offers high sensitivity, but limited specificity, for distinguishing between IBMFS and acquired bone marrow failure. Unaffected individuals—particularly family members of affected individuals— or individuals with other causes of bone marrow failure may also demonstrate telomere shortening. Alternatively, the natural histories are little known for individuals with similar clinical findings but less severe telomere shortening (e.g., Clericuzio-type poikiloderma). In a patient with marrow failure, however, the finding of normal telomere length obviates the diagnosis of DC *(2)*. Thus, no single approach—clinical, sequencing, or telomere length—is adequate as a stand-alone method. As high-throughput genomics penetrates clinical diagnostics, a more comprehensive genetic characterization of these patients should become standard practice.

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