A previously healthy 16-year-old boy was referred to our hospital for evaluation of bone marrow failure. He was noted to have an abnormal blood count when he was hospitalized for treatment of a femur fracture following a baseball injury.

The patient had hemoglobin of 12.9 g/dL with mean corpuscular volume (MCV) of 108, white blood cell count of 3,500/mm$^3$ with a normal differential, and platelet count of 60,000/mm$^3$. Bone marrow biopsy revealed a hypocellular marrow with dysplastic changes. Interphase fluorescent in situ hybridization (FISH) studies revealed monosomy 7 in 4% of the cells. Studies for paroxysmal nocturnal hemoglobinuria and Fanconi anemia were negative. He was referred to Lurie Children’s Hospital for consideration for allogeneic stem cell transplant.

Robert Listernick, MD, moderator:
Aside from the obvious cytopenias, what’s the significance of the elevated MCV?

Robert Liem, MD, pediatric hematologist: There was no obvious reason for him to have a nutritional macrocytic anemia due to vitamin B12 or folate deficiency. This is termed “stress hematopoiesis” because the bone marrow is trying to push out as many red blood cells as it can. The younger cells tend to be bigger even though the reticulocyte count is low. Hemoglobin electrophoresis performed in marrow failure syndromes often shows elevated levels of fetal hemoglobin that is in these “younger” cells.

Dr. Listernick: Is it fair to use the term “marrow failure” to describe this child’s blood count?

Dr. Liem: People throw around different terms. Although his hemoglobin is close to normal for age, he is leukopenic and thrombocytopenic and has a markedly elevated MCV. Given the bone marrow biopsy results, I believe that marrow failure is a reasonable description. The differential diagnosis is quite broad, including a variety of congenital and acquired syndromes. Vitamin B12 deficiency is considered the great masquerader that can cause this clinical picture. Even though he is a teenager, one has to consider all the constitutional conditions that might predispose to marrow failure, such as Fanconi anemia, Shwachman-Diamond syndrome, Diamond-Blackfan syndrome, and paroxysmal nocturnal hemoglobinuria.

Dr. Listernick: He was referred for stem cell transplantation. How does the monosomy 7 genetic abnormality factor into the equation?

Jennifer Schneiderman, MD, pediatric oncologist: The presence of a genetic abnormality such as monosomy 7 in the presence of a hypoplastic bone marrow shows that there is a clonal disorder in his bone marrow. Loss of chromosome 7, either the whole chromosome or the long arm, is the most common cytogenetic abnormality seen in childhood myelodysplastic syndromes (MDS). Approximately 20% of patients who have monosomy 7 go on to develop acute myelocytic leukemia. Therefore, once the presence of monosomy 7 is documented in a patient who has MDS, we...
recommend stem cell transplantation.

**Dr. Listernick:** What if the cytogenetics were normal in a patient with MDS?

**Dr. Schneiderman:** If a child has aplastic anemia without dysplastic changes, their treatment depends on their transfusion requirements. In patients who do not require regular transfusions of red cells or platelets, we generally recommend a program of “watchful waiting” with bone marrow testing yearly. In those who are transfusion dependent, patients with an HLA-identical sibling will move straight to transplant. Those patients who do not have an HLA-identical sibling undergo immune suppressive therapy with cyclosporine and antithymocyte globulin to assess for response. Children who don’t respond to therapy are referred for transplant. This child was not anemic and only required one platelet transfusion, which was given “prophylactically” prior to surgery to repair the fracture sustained while playing baseball before he was referred to us.

**Dr. Listernick:** What happens to these children who are simply watched?

**Dr. Liem:** We certainly have a cohort of older children with marrow failure who have a negative genetic work-up who we continue to follow long-term without any intervention other than intermittent transfusions. However, it’s important to follow them closely, as a significant proportion will convert to MDS and require stem cell transplant. If any of these children become significantly transfusion dependent, we might consider immunosuppressive therapy, which includes corticosteroids, cyclosporine, and antithymocyte globulin.

**Dr. Listernick:** Why does the loss of chromosome 7 predispose one to the development of MDS or leukemia?

**Katrin Leuer, PhD, cytogeneticist:** That’s a hot topic of research. For years, we’ve tried to identify candidate genes on chromosome 7 that may be responsible for this. There are two different regions on the long arm of chromosome 7 that have been identified but the exact genes are still unknown.

**Dr. Listernick:** Moving on, in preparation for stem cell transplantation, a repeat bone marrow examination was performed. Reading from the report, there was “hypocellular bone marrow, reduced multi-lineage hematopoiesis and relative erythroid hyperplasia with mild megaloblastoid maturation, but no increase in blasts. This is concerning for hypocellular MDS. No cytogenetic abnormalities were identified.” So, he doesn’t have monosomy 7? What happened?

**Dr. Schneiderman:** This was totally unexpected and we were only several days from starting chemotherapy in preparation for transplant. This is exactly the reason we always repeat a bone marrow biopsy, in case things have changed or if the original interpretation was wrong. We put on the brakes and reassessed the situation.

**Dr. Listernick:** Why the discrepancy?

**Dr. Leuer:** We obviously don’t know but can hazard several guesses. First, we made sure that there had been no mix-up of the samples that had occurred. We identify monosomy 7 using FISH, looking for two copies of chromosome 7. We repeated the FISH test on a separate cell culture and it was negative once again. Without going into the technical details, the outside sample was inadequate for karyotyping (mainly because of the marrow hypoplasia) and the FISH study was of poor quality. Despite the poor quality, the test was analyzed and interpreted. This situation ultimately led to a change in procedure in the outside lab. Fortunately, it was picked up here before transplantation was started.

**Dr. Listernick:** So, as I understand it, more startling news came back the same day.

**Dr. Schneiderman:** Yes. As part of his pre-transplant evaluation, he had been referred to the sperm bank at Northwestern Memorial Hospital. We have a very active fertility preservation program. On the same day that I received the cytogenetic results, I received a call that he had azospermia.

**Yasmin Gosiengfiao, MD, pediatric oncologist:** Almost all of the patients who undergo myeloablative stem cell transplantation become infertile. Any male 13 years or older is referred for sperm banking. Older teenage girls and adult women are offered oocyte cryopreservation, which is now standard of care. If they have a partner, they can also offer embryo cryopreservation. Although the data on success rates for pre-pubertal girls are not known, we are studying the use of ovarian tissue cryopreservation all the way to the neonatal period.

**Dr. Listernick:** What can you offer pre-pubertal boys?

**Dr. Gosiengfiao:** There are three or four institutions that offer testicular tissue cryopreservation; we’re in the process of trying to open a protocol here in coordination with the University of Pittsburgh.
Dr. Listernick: Retrospectively, he was noted to have “smallish” testes.

Donald Zimmerman, MD, pediatric endocrinologist: The problem is that pre-pubertal boys don’t have mature sperm. What’s interesting is that here’s a youngster who presumably has a normal amount of pubic hair, which suggests that he probably has normal levels of testosterone. However, at the same time, he has small testes. The Leydig cells that produce testosterone appear to be functioning normally, but the Sertoli cells that nurture the sperm are not.

Dr. Listernick: We obviously don’t deal with this problem frequently in pediatrics. What would be your approach to the evaluation of azospernia in a teenager, forgetting for a moment his MDS?

Dr. Zimmerman: It can be a pituitary, hypothalamic, or testicular problem. One would need to know the levels of testosterone, luteinizing hormone, and follicle-stimulating hormone (FSH). He had normal testosterone and luteinizing hormone levels but elevated FSH. This suggests that the pituitary, hypothalamus, and Leydig cells are functioning normally. Elevated FSH shows that the Sertoli cells are not working normally to support sperm. It all fits the clinical picture because Sertoli cells and sperm make up the bulk of the testes.

Dr. Listernick: Differential diagnosis?

Dr. Zimmerman: There are a number of conditions that could cause male infertility seen in pediatrics, including Klinefelter syndrome, cystic fibrosis, and autoimmune testicular failure. We wondered about this last diagnosis because he had recently been found to have mild hypothyroidism.

Dr. Listernick: What about in association with MDS?

Dr. Zimmerman: Nothing straightforward comes to mind. There’s a stretch of a diagnosis but I don’t want to go there yet.

Dr. Leuer: Just to interject a thought. We wondered about Klinefelter syndrome because many of these men can be mosaics. Our typical bone marrow analysis only looks at 20 cells. When we do an evaluation for possible Klinefelter mosaic disorder we look at 45 cells. All this testing was negative.

Dr. Listernick: Now what?

Dr. Liem: We went back to the drawing board. We rethought our approach to the evaluation of constitutional marrow failure. Part of the problem with the evaluation is that nobody has a good algorithm for how to look at all of the different candidate genes that might be implicated in marrow failure. There’s no panel of genes. Basically, we approach this type of testing sequentially, starting with the most likely candidate gene based on the signs and symptoms. The one bone marrow failure syndrome that came to mind in the context of azospermia was Fanconi anemia.

Dr. Listernick: How do you test for this?

Dr. Liem: Rather than gene testing, we started with chromosome breakage studies, which were negative. We even did a skin biopsy to culture fibroblasts looking for mosaic Fanconi anemia. It was all negative. We followed this with genetic testing for Schwachman-Diamond syndrome and testing for paroxysmal nocturnal hemoglobinuria, which was negative.

Dr. Listernick: What next?

Dr. Liem: As a group, we had intense discussions regarding this young man. Someone suggested we look at telomere lengths. There’s been a lot of literature suggesting that telomere biology is very important in marrow failure syndromes and it has some prognostic implications. His telomeres were below the first percentile in length — they were extremely short.

Dr. Leuer: Telomeres are the ends of chromosomes and are made up of
thousands of repeat sequences. My analogy is that they are like the plastic cap at the ends of your shoelace that keeps it intact; if they break, the chromosome starts to frazzle and lose its integrity. Maintaining telomere length is very important to maintenance of cell stability. It’s known that telomeres shorten in cancer.

**Dr. Liem:** I immediately contacted the National Institutes of Health (NIH) and was told categorically that this child has dyskeratosis congenita (DKC). Even though the *DKC* gene is on our list of candidate genes when looking for a cause of marrow failure, it’s often at the bottom unless there are specific signs. As I learn more about it, I now realize that there are many genes that are implicated in DKC and that penetrance may be variable.

**Dr. Listernick:** What is DKC?

**Dr. Liem:** DKC is a marrow-failure syndrome with predisposition to malignancy that is generally characterized by three classic symptoms: a reticulated and pigmented rash, dysplastic nails, and leukoplakia. The patient has none of these findings. The problem is that there are multiple genes that could lead to DKC with multiple phenotypes, some milder than others. Varying manifestations of DKC also include pulmonary fibrosis, liver disease, and variable subtypes of immunodeficiency with recurrent infections. *DKC1* is X-linked; mutations in this gene lead to the classic phenotype. There are also autosomal recessive and dominant genes that can lead to varying phenotypes. He has a heterozygous mutation in the *TERT* gene. The *TERT* and *TERC* genes are components of the machinery that keep telomerase lengths intact.

**Dr. Listernick:** What about treatment?

**Dr. Liem:** The marrow failure isn’t necessarily amenable to immunosuppressive therapy or the way that we treat typical aplastic anemia. Most of these children undergo transplantation. However, they may have a lot of problems with toxicity from the preparatory regimen, necessitating great care.

**Dr. Listernick:** Do they get second malignancies as a consequence of immunosuppression?

**Dr. Liem:** Yes. One of the biggest issues with DKC is that these children are at very high risk for not only MDS and other hematologic malignancies, but also squamous cell carcinoma of the mouth. They need annual visits to a good oral surgeon to look for early signs of leukoplakia.

**Dr. Zimmerman:** Telomerase dysfunction not only affects stem cells in the bone marrow but also takes its toll on the germ cells, which may account for the azospermia.

**Dr. Liem:** The NIH doesn’t follow anyone who has azospermia that they know of, but they are following several infertile individuals. There’s really nothing in the literature about this association.

**Dr. Listernick:** This is an autosomal disorder. What about the rest of the family?

**Dr. Liem:** We’re going to test both his parents and his siblings.

Barbara Burton, MD, pediatric geneticist: This case is a great example of why we’re moving more often toward whole exome sequencing. You made a great diagnosis that could easily have been missed. We’re beginning to learn of the much broader phenotypic variability in many of these genetic disorders, making whole exome sequencing the perfect test for some bizarre diagnostic dilemmas.

**Dr. Listernick:** Thank you, everyone.