Testing the Tests

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Clinical Presentation: A 5-year-old girl of Ashkenazi origin was referred to our pediatric endocrine clinic because of short stature. The child generally had good health aside from several episodes of otitis media. She was born at term after a normal pregnancy and delivery; birth weight was 2400 g, and birth length was not recorded. There was no history of intrauterine infection, toxemia, or exposure to alcohol or drugs. Developmental milestones were achieved at appropriate chronologic ages. The father’s height was 165 cm (third percentile); his first shave was at age 15 1/2 years. Maternal height was 157 cm (25th percentile); menarche was at age 12 years. Family history was unremarkable. The child’s height was 92 cm, and her weight was 10 kg (both below the third percentile). She was growing consistently below the third percentile; the upper and lower ratios were within the reference range. She had no dysmorphic features. Physical examination revealed no specific findings.

Dr Pinhas-Hamiel: A few factors might contribute to the child’s short stature. First, her birth weight is consistent with a diagnosis of intrauterine growth restriction. Long-term follow-up studies have shown that approximately 75% of children with intrauterine growth restriction will have catch-up growth within the first 2 months of life and that 85% will catch up by age 2 years. The remainder stay below the third percentile. Second, the midparental height is within the third percentile. Therefore, the child is growing according to her genetic potential and, moreover, at a normal velocity, which is an important variable in the evaluation of children’s health. These factors are sufficient for understanding the etiology and pathogenesis of her condition and for determining management. However, screening of apparently healthy children has shown that in approximately 15% of those who were growing consistently below the third percentile, there was an organic reason for short stature. Thus, systemic disease and endocrine dysfunction need to be ruled out. The patient’s bone age should be determined.

Physical Examination: Routine laboratory tests revealed a white blood cell count of 7.1 × 10^3/µL and a normal differential count, a hemoglobin level of 11.9 g/dL, a platelet count of 260 × 10^3/µL, and a sedimentation rate of 17 mm/h; renal function, liver enzyme, and thyroid function test results were within the reference ranges. Antigliadin antibodies were negative. Urine test results were normal. Bone age was compatible with 3 1/2 years.

Dr Pinhas-Hamiel: The possibility of a silent disorder that may be expressed solely by impaired growth, such as inflammatory bowel disease or celiac disease, is unlikely in the presence of normal blood test results. There is no family history of delayed puberty, but the young bone age compared with the chronologic age suggests that constitutional delay may be an additional contributing factor to the short stature. Finally, although there are several possible explanations for the short stature in this patient, every female growing below the third percentile should undergo chromosomal analysis to rule out Turner syndrome.

Physical Examination: A chromosome analysis was performed on cultured lymphocytes from peripheral blood samples. The preparation was treated with trypsin to obtain G-banding according to standard techniques. Karyotype established after the analysis of 20 metaphases revealed a healthy girl with 46,XX.

When the child was 6 years old, she began to complain of recurrent headaches. Ophthalmologic examination showed normal visual acuity and optic fundi. There were no abnormal neurologic findings. Skull radiographic evidence was normal. Her prolactin level was normal, and her incidental growth hormone level was 14 ng/mL (normal, >10 ng/mL). The headaches resolved spontaneously.

Dr Pinhas-Hamiel: In the clinical context of short stature and headaches, the possibility of central nervous system involvement should be considered. I generally do not perform incidental growth hormone measurements, as the secretion of growth hormone is episodic, and random blood samples are frequently not informative. When growth velocity is abnormal, I prefer provocative stimulation tests. Nevertheless, the normal growth hormone level in this case rules out growth hormone deficiency. The contribution of skull radiographic evidence is limited, and, currently, when there is a clinical suggestion, other brain imaging studies are performed. With a normal serum level of growth hormone, no evidence of central nervous system insult, and a normal karyotype, the major possible diagnoses have been excluded. I would have the child undergo yearly height measurement by the pediatrician to affirm that she is growing consistently and to reassure the family.
Physical Examination: The child was followed for the next several years. She continued to grow below the third percentile, with a gap of approximately 2 years between her chronologic and bone ages. At age 11 years, breast buds and pubic hair developed. At age 13 years and 8 months, breasts were Tanner stage IV, with no menses, and bone age at that time was compatible with 12 years.

Dr Pinhas-Hamiel: The child has normal puberty and physical development. I am not concerned about the lack of menses. In the presence of secondary sex characteristics, primary amenorrhea is not considered until 16 years of age. As her bone age is young, she still has potential to grow.

Clinical Presentation: The patient returned to the clinic when she was 16 years and 7 months old. Menarche had occurred at age 14 years, and the second menstruation at 15 years 2 months; since then, she had not had any periods. Blood pressure was 95/55 mm Hg, height was 143 cm, and weight was 32 kg (much less than the third percentile). She had not gained weight since age 13 years. She denied intentional weight loss, and there were no behavioral signs suggesting pursuit of thinness. She was doing well at school and had peer relationships. She was not sexually active. There was no history of constipation, fatigue, or galactorrhea, but she did notice hot flashes. There were no signs of hirsutism or acne.

Dr Pinhas-Hamiel: A lack of periods for 18 months after menarche or cessation of periods for more than 6 months is consistent with secondary amenorrhea. Amenorrhea and weight loss, or lack of weight gain, during puberty are warning signs of an eating disorder. Adolescent patients will often deny efforts to lose weight. Because of the patient’s low weight and amenorrhea, gastrointestinal tract disorders should be checked for again. Highly sensitive and specific markers of celiac disease are now available. Endocrine evaluation should include levels of gonadotrophins, estrogen, prolactin, and adrenal hormones. Specifically, polycystic ovary disease and late-onset 21-hydroxylase deficiency can present with menstrual irregularities. Although hirsutism is usually a clinical sign, it is often hard to detect as adolescent girls may use cosmetic procedures. Therefore, I generally measure testosterone and 17-hydroxycortico-steroid progesterone levels as well.

Physical Examination: Antiendomysial and transglutaminase antibodies were negative. Serum levels of iron and folic acid and sedimantation rates were normal, as were levels of adrenal hormones, testosterone, and prolactin. The luteinizing hormone level measured 142 mIU/mL, and the follicle-stimulating hormone level was 77 mIU/mL (reference ranges in menstruating females: up to 22 and 17 mIU/mL, respectively). Her estrogen level was less than 6 pg/mL.

Dr Pinhas-Hamiel: The elevated levels of the gonadotrophins and the low level of estrogen are consistent with primary ovarian failure and not with the hypothalamic amenorrhea of anorexia nervosa. Ovarian failure occurs in approximately 3% of females younger than 30 years. Although usually idiopathic, it can be secondary to enzymatic deficiencies or an autoimmune process. Deficiency of 17α-hydroxylase and/or 17,20-desmolase should be considered as a rare cause of hypergonadotropic hypogonadism. The deficiency of the enzyme results in increased production of progesterone deoxycorticosterone and corticosterone. Hypertension may be variable; however, affected patients grow somewhat tall, and adrenarche is lacking. Autoimmune oophoritis can be either isolated or part of a systemic autoimmune disease such as type 1 autoimmune polyglandular disease or autoimmune Addison disease. Other causes include radiotherapy, chemotherapy, surgery, viral diseases, and chromosomal disorders.

Physical Examination: Ultrasound of the pelvis revealed a normal uterus measuring 37 × 19 × 29 mm. The endometrium measured 13 mm, and the ovaries measured 23 × 15 and 20 × 16 mm, with few follicles. Anti-ovarian antibodies were negative. The progesterone level was normal. Administration of 250 µg of synacthen to rule out Addison disease7 revealed adequate increase of plasma cortisol levels.

Dr Pinhas-Hamiel: We now have a female with short stature and premature ovarian failure whose other endocrine functions are within the reference limits. Although her karyotype was reported to be normal, at this point I would repeat the test or perform a skin biopsy to check fibroblasts. Patients with Turner syndrome may have mosaicism that is not generalized but limited to selected organs. This may lead to misdiagnosis if a biopsy specimen from an unaffected organ is obtained for karyotypic analysis.

Physical Examination: Repeated karyotypic analysis revealed a mosaicism of 80% 46,XX and 20% 45,XO, consistent with the diagnosis of Turner syndrome. The patient had normal findings on kidney ultrasound and echocardiography; bone mineral density was also normal. She began estrogen replacement therapy, and her hot flashes stopped.

Dr Pinhas-Hamiel: Chromosomal variants may result in a spectrum of clinical features ranging from full-blown Turner syndrome to virtually no somatic abnormalities. The single most common clinical finding in patients with Turner syndrome is short stature. Affected patients may also experience intrauterine growth restriction, lymphedema of the hands and feet, webbing of the neck, a shieldlike chest, recurrent upper respiratory tract infections, cardiac and renal malformations, developmental delay, and gonadal dysgenesis. Approximately 2% of 45,XO and 12% of mosaic women with Turner syndrome have sufficient residual follicles to allow for spontaneous breast development and menstruation; however, they will often have premature menopause.

Parents are often concerned about the height of their children, as children with short stature are believed to have low self-esteem and are frequently laughed at. Growth

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problems are the nitty-gritty of pediatric practice. However, it is not the cosmetic aspect of being short that concerns pediatricians but the fact that growth is a sensitive indicator of general health. As even minimal aberrations in health may be reflected in a deviation from the normal growth rate, growth should be carefully followed, and a workup should be performed when a deviation is found.

Of the various causes of short stature, Turner syndrome is relatively common, occurring in 1 of 5000 to 5000 female births. When patients present only with short stature, it is often hard to reach the correct diagnosis early, resulting in delay in treatment and detection of comorbidities. The number of years of growth hormone therapy is a significant determinant in improving final height.

A 45,XO karyotype accounts for approximately half of all cases of Turner syndrome. The remainder have either mosaic karyotype or structural abnormalities of the X chromosome. The detection of mosaicism depends on the type and number of tissues analyzed, the number of cells studied, and the sensitivity of the technique applied. Because many cells in metaphase have to be analyzed to detect the second cell line, a small percentage of mosaicism may not be detected by conventional cytogenetic techniques. Results have improved considerably with the recent introduction of molecular techniques such as fluorescence in situ hybridization and polymerase chain reaction.

The diagnostic process must always start and end with the patient. We observe the patient’s symptoms and signs and formulate hypotheses regarding the nature of the illness. We then perform tests and conduct further investigations, and we modify our hypotheses according to the results. The more information we gain, the lower our level of uncertainty regarding the diagnosis and, hence, the optimal treatment. In the present case, the physician asked for a karyotype examination at the initial evaluation, although at that time no clinical signs suggesting Turner syndrome were evident and there were several other acceptable explanations for the short stature.

In modern medicine, we tend to rely more and more on laboratory tests and imaging studies and less on our clinical skills, especially when clinical symptoms are scarce. Although tests cannot make us 100% certain of a diagnosis, they can help narrow the possibilities in a pragmatic manner. Therefore, in the present patient, the physician took the initial karyotype results at absolute value. The possibility of a false-negative finding never occurred to her.

When tests are essential to the diagnosis, we need to know their sensitivity, specificity, and likelihood ratios so that we can rely on them. Their value lies on the method itself (variation in kits, technical performance) and on our interpretation of the results. The errors and risks inherent in diagnostic tests demand that we consider them in terms of probability and not as absolute information.

When we get a positive test result, such as a positive finding from the biopsy specimen from a breast lump, a multidisciplinary team comprising a pathologist, a surgeon, and an oncologist will gather to review the findings. However, perhaps because we as physicians very much want the results to be negative, when they are, we rarely question their validity. Nonetheless, daily life proves that the acceptance of a negative result requires great awareness and caution. In the present case, even when more information was gathered over time showing a clinical history of secondary amenorrhea, the discussant considered anorexia and gastrointestinal tract problems, but she did not question the reliability of the karyotype testing.

This case demonstrates that a high index of suspicion is needed to pursue clinical hunches even when tests do not support the suspected diagnosis. Wise use of a test requires analytic evaluation of its diagnostic abilities. We should always bear in mind that we diagnose patients, not their test results.

Accepted for publication August 7, 2002.

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