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The impact of assay sensitivity in the assessment of diseases and disorders in children

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ABSTRACT

Accurate measurement of the low levels of testosterone (T) and estradiol (E_2) present in normal children and in children with disorders of puberty and sexual development is critical both for appropriate diagnosis and treatment and for clinical research studies. However, measurement of these levels lacks needed precision because of inadequate sensitivity of most commercially available assays and poor accuracy at the low levels found in normal childhood and most disorders. While immunoassays presently do not appear to have the potential to provide more accurate measurements, isotope dilution-gas chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry techniques offer promise to meet this need to improve clinical care and research.

1. Introduction

The development of the radioimmunoassay for sex steroids marked a very significant milestone for the diagnosis and clinical investigations of normal physiology and pathophysiology of sexual development during childhood and adolescence. The initial assays involving extraction and column or paper chromatography followed by radioimmunoassay had specificities and sensitivities allowing reasonable discernment of the levels of sex steroid. Subsequent development of better antibodies with much less cross-reactivity, together with the need to reduce the labor intensity, cost, and turn around time involved in these assays, led to the development and utilization of assay kits using a platform system. However, the resultant sensitivity is generally not greater than that attained previously, and in some instances there are poorer lower limits of detection and variability of results for the low concentrations typically found in children and adolescents.

More accurate measurement of serum gonadal steroid concentrations, however, is crucial to attain the needed and desired level for current state-of-the-art diagnosis and care of the child since current sensitivity is frequently inadequate to accurately diagnose many pediatric disorders. Currently available immunoassays often do not provide accurate enough results in children, who have lower circulating levels [1]. While test methods are validated for adults who have higher values,

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they are commonly inadequate for both clinical and research purposes in children. In order to optimize clinical diagnosis, monitor therapy and conduct meaningful clinical research concerning normal and abnormal development among children, more accurate and reproducible values are mandatory.

This review is limited to assays of estradiol (E_2), primarily of pertinence among girls, and testosterone (T), more often applicable to boys. Standard metric units are used throughout, but conversion factors to SI and other commonly used units are provided in the sidebar. The frequently cited lower limit of detection (sensitivity) of T is often reported as 10 ng/dl. However, if one compares several commercially available third generation immunoassays, the same sample may be reported as values ranging from below the detection limit to values ranging several-fold greater than this lower limit. Since the level of circulating E_2 in the prepubertal child is considerably less than T, the issues of sensitivity and being able to obtain a clinically useful value are even greater.

Steroid	Conversion from standard metric units		
	To convert:	To:	Multiply by:
Testosterone	ng/dL	nmol/L	0.03467
	pg/mL	pmol/L	0.03467
	ng/dL	pg/mL	0.1
	pmol/L	ng/dL	0.0288
Estradiol	pg/mL	pmol/L	3.671
	ng/dL		36.71

Examples of misleading values of these steroids further illustrate the added expense and difficulty reaching a diagnosis among children, together with additional complexity of the medical assessment and stress for parents already having an infant with significant problems.

Such an example involves a child who presented with genital ambiguity who was initially evaluated at 3 days of age with a karyotype and sex steroid levels, commonly assessed in the child presenting with disordered or ambiguous genitalia. The karyotype was 46,XX and the phenotype was recognized as that typical of cloacal exstrophy, but an initial T value was reported to be 200 mg/dL. Such an elevated value suggested that assessment of the patient should involve a search for a source of androgen and potential differentiated testicular tissue with differentiated Leydig cells, a situation which was not part of the differential diagnosis of a 46,XX child with cloacal exstrophy. The initial high value may have been a consequence of cross-reacting steroids or other interfering substances circulating in a neonate. A specific assay would have avoided this problem.

Much more common are the examples of variation in T levels in the range below 50 ng/dL complicating the assessment of pubertal status among males of pubertal age. A very common clinical question among boys with late onset of puberty is whether puberty has begun and whether the patient has the potential for normal hypothalamic and pituitary axis function. Typically, a 14-year-old boy presenting with a chief complaint of delayed puberty has a physical examination with subtle changes that might represent the onset of puberty, including a few pubic hairs, genitalia of late prepubertal or early pubertal size, and testicular volume of 4 cc. The differential diagnosis in such a boy, after determination that gonadotropins are not elevated, ruling out testicular failure and primary hypogonadism, is between constitutional delay of puberty and hypogonadotropic hypogonadism. Often the initial measurement of T in such a patient approximates 35 ng/dL. However, because of variability of the assay toward the lower limits of sensitivity, such a value is not helpful, because the "true" level may range from undetectable to 50 ng/dL. If, using a reliable assay for T, a measurement of 45 ng/dL is reported, this would be indicative of the onset of testicular T secretion. Conversely, a level of <10 ng/dL would mean no pubertal T secretion. However, using commonly available T assays, such a differential cannot be made until the passage of more time, when the patient clearly does or does not demonstrate pubertal T secretion.

A converse example among males is the presentation at age 8 years with a complaint of early onset of puberty. In such a boy, the common presentation, involving minimal sexual hair and borderline testicular and penis size, poses a similar problem, with the accuracy of generally available T assays failing to discern levels characteristic of pubertal onset from prepubertal levels.

Thus, since prepubertal values may range from 10 to 40 ng/dL using available T assays, sensitivity is not adequate to differentiate prepubertal and pubertal secretion. With a sufficiently reliable assay, the range of levels for prepubertal and pubertal males could be established to differentiate these states.

A similar case can be made for the relative lack of usefulness of current E_2 assays. The only situation in which E_2 measurements are clearly efficacious is in assessment of estrogen secreting tumors. A sensitive assay, capable of discerning values well below 15 pg/mL [2] down to 5 pg/mL or lower, would be useful in the diagnosis of precocious puberty and delayed puberty, to differentiate precocious puberty from premature thelarche, and to ascertain adequate suppression of central precocious puberty with gonadotropin-releasing hormone analogs (GnRHa).

2. Laboratory testing of gonadal steroids in children

As the above discussion shows, the accurate measurement of the low levels of T and E_2 seen in normal children and in children with disorders of puberty and sexual development is critical for appropriate diagnosis and treatment. Unfortunately, the measurement of these levels is fraught with difficulty. This difficulty arises both in terms of the inadequate sensitivity of most commercially available assays, particularly those for E_2, and in very low accuracy and reproducibility at low levels for both T and E_2.

The assays typically used in most hospital and free-standing commercial laboratories were designed and validated for use in adult males (T) and females (E_2). However, medical decision making involving infants and children may require measurement of sex steroid concentrations 100-fold