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Editorial

The Art of Hormone Measurements with Emphasis on Specificity Resulting in Diagnostic and Management Improvements

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Editorial

For over 30 years, our laboratory has dedicated research efforts toward improving the accuracy of diagnostic testing. Our research program originates from key interactions with the former head of endocrinology and thyroid expert at Children's National Medical Center, Dr. Wellington Hung. Dr. Hung called attention to a critical problem occurring in thyroid function testing: the results for Free Thyroxine (FT4) measured by Immunoassay (IA) often did not agree with values obtained for Thyrotropin Stimulating Hormone (TSH) nor with the patient's clinical condition, especially in cases where TSH was elevated. In other words, FT4 testing by IA was frequently disagreeing with the clinical diagnosis possibly resulting in inappropriate treatment of hypothyroidism in children. Therefore, accurate diagnostic testing is paramount.

There are many factors that need to be considered when evaluating the accuracy of diagnostic tests. The most obvious concern has to do with the specificity of the testing mechanism employed. While direct IA measurements for small molecule analytes are convenient, relatively economical, and precise [1], they are also prone to cross-reactivity and competitive binding interactions which can lead to inaccurate results [2,3]. Cross-reactivity has been well-characterized in steroid hormone measurements, where antibodies are unable to differentiate among numerous biologically distinct hormones with a shared molecular scaffold [4]. Competitive binding interactions, are often alluded to in the literature, but rarely addressed directly [5,6]. Recently, our laboratory found that naturally occurring abnormal fluctuations of specific binding proteins (Thyroxine Binding Globulin (TBG), Corticosteroid Binding Globulin (CBG)) affected the accuracy of total and free Triiodothyronine (TT3 and FT3) and cortisol measurements by IA [7,8]. By contrast, measurements of FT4, FT3, TT3 and cortisol by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) correlate accurately with the clinical presentation [8-12]. Assays by LC-MS/MS typically require a protein precipitation, or purification step (dialysis, ultrafiltration) during sample preparation which physically removes competing binding proteins from the sample. Additionally, analyte identification and quantification is determined by the detection of specific masses resulting from unique fragmentation patterns, rather than binding affinity. Thus, LC-MS/MS methods have greater specificity than their IA counterparts, and should be the preferred method for evaluating thyroid and adrenal function among other important steroid profiles.

Another important, yet often overlooked, factor to consider when assessing diagnostic results is whether the reference interval provided is appropriate. A recently published study [13] highlights the importance of precision medicine in the definition of pediatric thyroid function test reference intervals, and brings forth necessary discussion of key issues surrounding the measurement of and diagnostic reliance on TSH that we believe may benefit the readership. In addition to the variables described by Oron et al. that affect TSH levels [14], steroid concentration must also be taken into consideration for the accurate reporting of TSH in thyroid function evaluation [10,11,14-18]. This effect was clearly demonstrated by our previous investigation in which we observed substantial decreases in TSH measurements after ACTH stimulation, when steroid concentrations are high [11]. It is important to remember that neonatal steroid hormone levels are much higher than those in adults, as newborns at birth have similar concentrations of estradiol and progesterone to their mothers [19]. Neonatal screening programs usually occur between days 2-5 postpartum. Higher steroid hormone levels would lower TSH serum levels in newborns, potentially leading to falsely normal values of TSH, masking an underactive thyroid. This scenario is particularly concerning as undiagnosed hypothyroidism in newborns can lead to serious permanent deficiencies in neurocognitive development [20]. While the incidence of congenital hypothyroidism may be considered rare (1 in 3000 births) [21], the long-term effects of a missed diagnosis are of great cost to the patient. At present the measurement of TSH is the primary screening strategy used to detect and diagnose congenital hypothyroidism in newborns [22,23]. This recommendation needs to be revisited given the common variation in serum TSH levels and often inaccurate reference intervals.

Many reference intervals for TSH are not accurate for the populations they serve-whether composed of different ethnicities or of patients with distinct steroid profiles. Hypothyroidism affects a significant population of patients, yet screening and diagnosis often rely heavily on TSH concentrations, which are variable, especially depending the concentration of steroid hormones present. Steroid hormone concentrations are clearly affected by age (over puberty and adolescence) and sex, and also by very commonly prescribed medications such as statins [24], oral contraceptives [18] and drugs

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to alleviate seasonal allergies [25]. For the past three decades we have implemented IA test reflexing for when measurements of TSH are either high or low, automatically sending samples for thyroid hormone measurements by Liquid Chromatography Tandem Mass Spectroscopy (LC-MS/MS). In our experience, TSH levels that are lower than 1mIU/L typically correspond to patients who are taking a regimen of steroids. We suggest that thyroid function assessments take into account more than just TSH. Experience has shown that IA's for TT3 correlate well with measurements of TT3 by LC-MS/MS and with the clinical picture at normal to high concentrations [10,11,26,27]. At low TT3 concentrations, however, this is not true. IA shows a positive bias of 30-40 ng/dl [28]. Of course, our advice for relying on these additional diagnostic markers requires that the appropriate reference intervals [29] also be established.

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Author Contribution(s)

Anastasia Gant Kanegusuku: Conceptualization; writing-original draft preparation; writing-review and editing.

Steven J. Soldin: Conceptualization; resources; writing-original draft preparation; writing-review and editing.

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