How Do We Measure Hyperandrogenemia in Patients With PCOS?

Brian G. Keevil

Department of Clinical Biochemistry, University Hospital of South Manchester, Manchester M23 9LT, United Kingdom; and Centre for Endocrinology & Diabetes, Institute of Human Development, University of Manchester; Manchester Academic Health Science Centre, Manchester M13 9NT, United Kingdom

The latest guidelines recommend that hyperandrogenemia should be evaluated biochemically in all women suspected of having PCOS (1). There is currently no consensus on what is the most appropriate androgen to measure or the upper cutoff consistent with PCOS, but it has been generally accepted until now that T is the most commonly ordered measurement for the investigation of female hyperandrogenemia. This concept is challenged by O'Reilly et al (2) in this issue of the *JCEM*. It has previously been reported that androstenedione (A) can be raised when T is normal in patients with hirsutism (3) and PCOS (4), but O'Reilly et al (2) now show that patients with high A and normal T concentrations have nearly as much risk for metabolic disease as those with a high T concentration alone. The results of this study raise concern that hyperandrogenemia may be missed if only T is measured. Importantly, the serum A and T results were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS). LC-MS/MS methods for measuring steroids represent the state of the art and can reliably measure accurate concentrations of A and T in female subjects. The concentration of T in females is 15 to 20 times lower than that found in males, and this has always posed a major challenge to "direct" immunoassay methods. Most clinical chemistry laboratories perform "direct" T immunoassays (without any extraction step) routinely on automated analyzers to improve the throughput and cost of analysis. Extraction steps using organic solvents had been popular and were very effective for removing interfering substances before analysis by immunoassay methods, but they are time-consuming and expensive in staff time. The extraction steps were discarded to streamline the process, but the resultant direct methods, although convenient, could suffer significant interference from other steroids such as dehydroepiandrosterone sulfate (DHEAS). DHEAS is the most abundant steroid produced by the adrenal gland and is present in 1000-fold higher concentrations than T, and the resultant positive interference increased inaccuracy of the result (5, 6). T measurement by direct immunoassay is also limited by a lack of sensitivity and specificity, which has been well documented and has prompted a recommendation from The Endocrine Society to avoid using such assays (7). In addition, the continuing poor performance of many of these direct assays has prompted a recent editorial in the *JCEM* (8), stating that LC-MS/MS is a mandatory submission requirement for sex steroid results as of January 2015.

A is not as widely measured in clinical chemistry laboratories as T. This is partly due to demand because A is often used as a secondary test to be ordered only when T is elevated and also because A is not routinely available on main clinical chemistry analyzers. The smaller niche immunoassay analyzers available for measuring A typically give results twice the concentration of those reported by LC-MS/MS methods (9). A consequence of reporting these higher immunoassay results is that the diagnostic cutoffs reported by O'Reilly et al (2) are not applicable, thus necessitating the need for separate assay-specific reference intervals. Whether properly constructed reference intervals applicable to specific populations exist for these assays is debatable. The argument for measuring A only on samples with high T results, usually to save money and resources when using immunoassay, is now flawed because it is clear that measuring A only when T was raised

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received February 3, 2014. Accepted February 7, 2014.

For article see page 1027

Abbreviations: A, androstenedione; DHEAS, dehydroepiandrosterone sulfate; LC-MS/MS, liquid chromatography-tandem mass spectrometry.

would have missed individuals with the high A-normal T phenotype. Although A is not a bioactive androgen itself, its importance lies in being the immediate precursor of T. It is secreted by ovaries and adrenals and can also be generated in peripheral tissues from its precursor dehydroepiandrosterone by the enzyme 3β-hydroxysteroid dehydrogenase type 2. Conversion of A to T occurs mainly in the ovary, but it can also occur in adipose and other peripheral tissues by the action of 17β-hydroxysteroid dehydrogenase type 5, which accounts for approximately 25% of T production (10). An enlarged pool of A may therefore act as a surrogate for metabolic complications because of its potential for further conversion into T. O'Reilly et al (2) point out that a discrepancy between A and T results may also result from the affects of hyperinsulinism on SHBG binding, which, while not affecting A, does lower the clearance and bioavailability of T.

Although O'Reilly et al (2) acknowledge that LC/MS-MS has dramatically improved the rapid detection and reliable quantification of serum steroids in both clinical and research practice, the technique is currently only available in larger institutions with sufficient expertise and resources. To highlight this point, the UK National External Quality Assessment Scheme (9) has 194 enrolled laboratories reporting female T results, only 27 of which use LC-MS/MS methods. In the same scheme, only 50 laboratories in total report A results, and 26 of these use LC-MS/MS, reflecting the number of laboratories also reporting T by LC-MS/MS. Why has there been such a poor take up of this technology in clinical laboratories? Undoubtedly, the technique is relatively expensive when compared to the low unit costs of a high throughput routine clinical analyzer, but a bigger problem lies in the need for highly skilled operators to run the instruments and prepare samples. The preparation of calibrators and reagents is an important issue because, unlike clinical analyzers that use fully prepared kits, these simply do not exist for many LC-MS/MS steroid assays. An important concern is that neat serum cannot be injected straight into an LC-MS/MS instrument, and some sample preparation needs to take place to clean up the samples. In the case of steroids, this usually entails a similar solvent extraction to that ironically sacrificed to improve the throughput of direct immunoassays. Despite these problems, a major advantage of LC-MS/MS over immunoassay is the capability for multiplex analysis, ie, T and A can be measured simultaneously at no extra cost; indeed, it is also possible to multiplex DHEAS and 17-hydroxyprogesterone within the same run so that significant ovarian pathology and nonclassical congenital adrenal hyperplasia can be investigated at the same time.

The Endocrine Society and the Centre for Disease Control (CDC) are making great strides in harmonizing steroid results between laboratories by providing reference serum samples with assigned values (11). These samples can be used to check and control at frequent intervals the calibration accuracy of laboratories. As mentioned previously, this is vital because laboratories are currently manufacturing their own standards and calibration materials. Currently, the CDC Laboratory/Manufacturer Hormone Standardization Program is only available for T and estradiol, but it would be essential for A to be included in such calibration initiatives to improve interlaboratory variability, thus enabling the use of common reference intervals and diagnostic cutoffs. It is hoped that both LC-MS/MS and immunoassay methods will benefit from these harmonization programs, but many of the problems with direct immunoassay arise from poor specificity of the antibody. It is therefore hard to see how these direct methods will improve as a result of improved calibration when they have underlying technical problems.

LC-MS/MS is a better measurement technique than immunoassay for steroid analysis because it does not suffer from cross-reactivity, the limit of quantification is lower, and it can multiplex more than one steroid. A possibility for future research may be the development of salivary T assays, using high-sensitivity LC-MS/MS instruments, which can measure the very low concentration ranges encountered in females (12). Initial results with this assay are encouraging and show good demarcation between male and female ranges, but there are as yet no reported results in PCOS patients.

Should we all be clamoring to measure serum androgens using LC-MS/MS? I believe the answer is emphatically yes, especially for the investigation of females with hyperandrogenemia, and particularly if we measure multiple androgens. The study of O'Reilly et al (2) should provoke and guide further research into the role of androgens in metabolic risk assessment in PCOS, hopefully with the use of well-validated, high-quality LC-MS/MS methods for steroid analysis. O'Reilly et al (2) make a strong case for routine serum A measurement in the assessment of PCOS, and we should follow their lead.

Acknowledgments

Address all correspondence and requests for reprints to: Brian Keevil, MSc, University Hospital of South Manchester, Clinical Biochemistry, Manchester M23 9LT, United Kingdom. E-mail: Brian.Keevil@UHSM.NHS.UK.

Disclosure Summary: The author has nothing to declare.

References

1. **Fauser BC, Tarlatzis BC, Rebar RW, et al.** Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertil Steril*. 2012;97:28–38.e25.
2. **O'Reilly MW, Taylor AE, Crabtree NJ, et al.** Hyperandrogenemia predicts metabolic phenotype in polycystic ovary syndrome: the utility of serum androstenedione. *J Clin Endocrinol Metab*. 2014;99:1027–1036.
3. **Duxbury K, Gallagher L, Keevil B.** The impact of simultaneous measurement of testosterone and androstenedione in women with suspected androgen excess. *Clin Chem*. 2007;53:804–805.
4. **Barth JH, Field HP, Yasmin E, Balen AH.** Defining hyperandrogenism in polycystic ovary syndrome: measurement of testosterone and androstenedione by liquid chromatography-tandem mass spectrometry and analysis by receiver operator characteristic plots. *Eur J Endocrinol*. 2010;162:611–615.
5. **Warner MH, Kane JW, Atkin SL, Kilpatrick ES.** Dehydroepiandrosterone sulphate interferes with the Abbott Architect direct immunoassay for testosterone. *Ann Clin Biochem*. 2006;43:196–199.
6. **Middle JG.** Dehydroepiandrostenedione sulphate interferes in many direct immunoassays for testosterone. *Ann Clin Biochem*. 2007;44:173–177.
7. **Rosner W, Auchus RJ, Azziz R, Sluss PM, Raff H.** Position statement: utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society Position Statement. *J Clin Endocrinol Metab*. 2007;92:405–413.
8. **Handelsman DJ, Wartofsky L.** Requirement for mass spectrometry sex steroid assays in the Journal of Clinical Endocrinology and Metabolism. *J Clin Endocrinol Metab*. 2013;98:3971–3973.
9. **United Kingdom National External Quality Assessment Service.** Birmingham Quality. www.birminghamquality.org.uk
10. **Quinkler M, Sinha B, Tomlinson JW, Bujalska IJ, Stewart PM, Arlt W.** Androgen generation in adipose tissue in women with simple obesity–a site-specific role for 17β-hydroxysteroid dehydrogenase type 5. *J Endocrinol*. 2004;183:331–342.
11. **Rosner W, Vesper H.** Toward excellence in testosterone testing: a consensus statement. *J Clin Endocrinol Metab*. 2010;95:4542–4548.
12. **Keevil B, MacDonald P, Macdowall W, Lee DM, Wu FC; the NATSAL Team.** Salivary testosterone measurement by liquid chromatography tandem mass spectrometry in adult males and females [published online November 5, 2013]. *Ann Clin Biochem*. doi:10.1177/0004563213506412.