Using FAI for Biochemical Evidence of Hyperandrogenaemia in Females: Mass Spectrometry vs Immunoassay Derived Results.

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Immunoassays have made significant improvements in the accurate measurement of female testosterone, however testosterone alone has limitations as a marker of hyperandrogenaemia. Testosterone results measured by either immunoassay (IA)or mass spectrometry (MS) are used in combination with SHBG to calculate Free Androgen Index (FAI) which has been shown to be a better marker of hyperandrogenaemia. This study compares FAI derived from both immunoassay and mass spectrometry measured testosterone results.

Aim – To establish if a significant difference exists in interpretation of FAI between mass spectrometry and immunoassay derived results

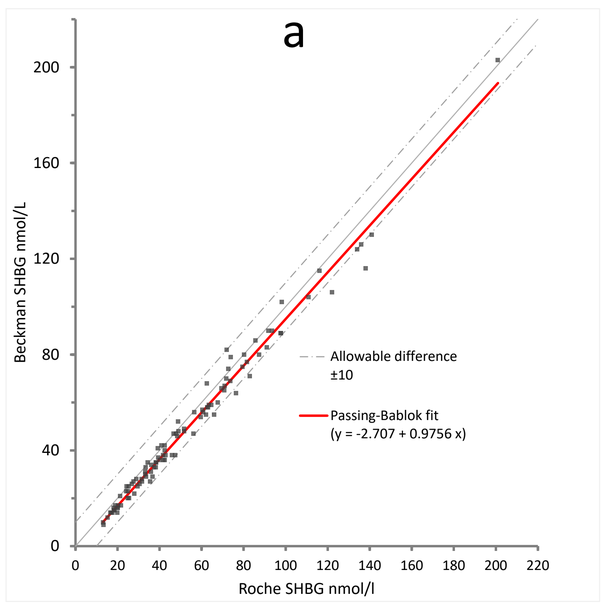
Methodology

The study cohort comprised of serum from females (n=106) which were routinely being tested for the assessment of hyperandrogenaemia. MS testosterone was measured on a Waters Quattro Premier mass spectrometer and converted to FAI in combination with a Beckman Access derived SHBG result and reported against a locally validated reference interval. Immunoassay testosterone and SHBG were measured by Roche, then converted to FAI and results were assessed against the manufacturers stated reference interval. Testosterone and SHBG assays were compared between the two respective methods and additionally classification of hyperandrogenaemia based on FAI was compared between the two systems used.

Results

SHBG results (Beckman vs Roche) were shown to be comparable with no significant difference between the two immunoassays (paired t-test: p=0.11). Testosterone results (MS vs Roche) showed a 25% negative bias for the Roche immunoassay. (fig 1a &1b) Converting results to FAI indicated a significantly higher incidence of abnormality, or biochemical evidence of hyperandrogenaemia, in the Mass spectrometry group vs the Roche IA group (n=37 vs 17). For the additional 20 patients with raised FAI detected by mass spectrometry, 20 had a testosterone within the immunoassay reference interval, although a low SHBG was apparent in 65% of these cases. SHBG was a more sensitive indicator of hyperandrogenaemia in this group (Fig 3)

Fig.1 Method comparison for (a) SHBG and (b)Testosterone



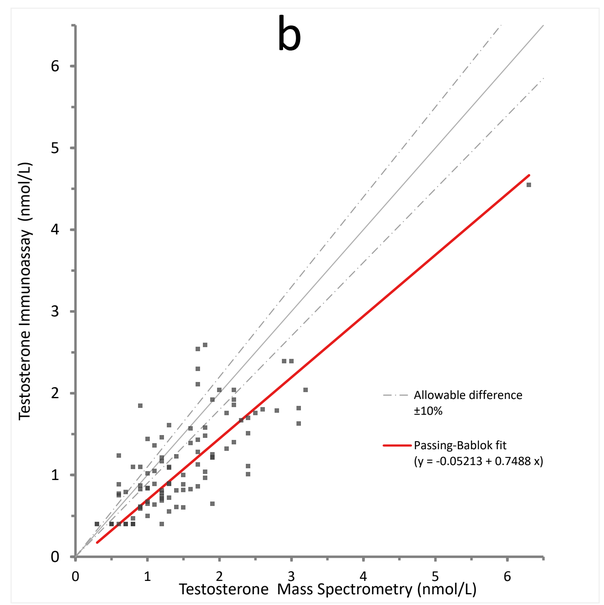
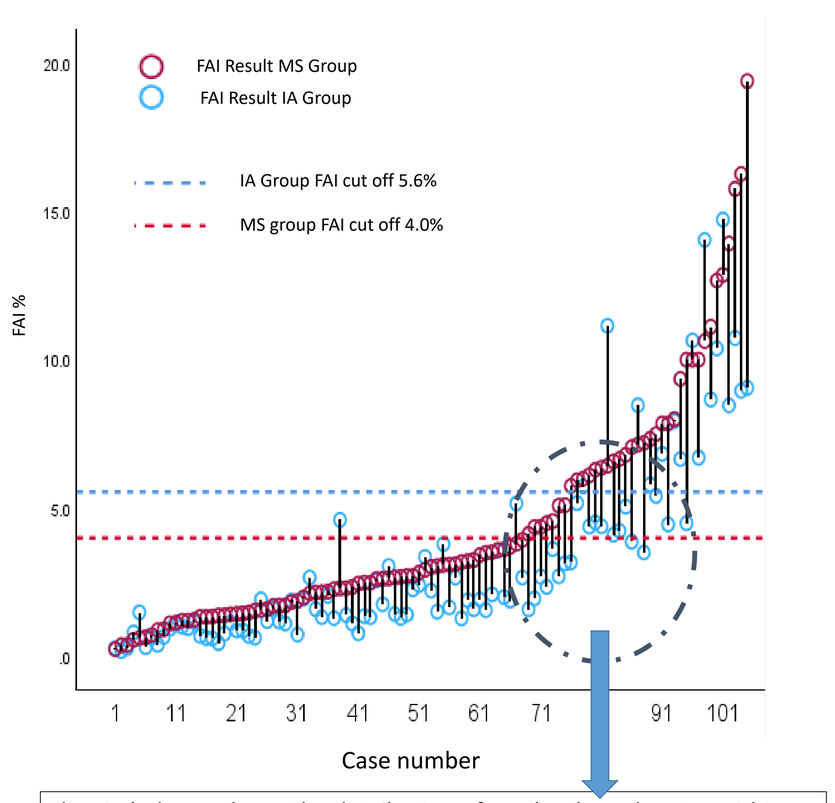


Fig 2 Discordance plot showing paired (linked)results for MS group vs IA group

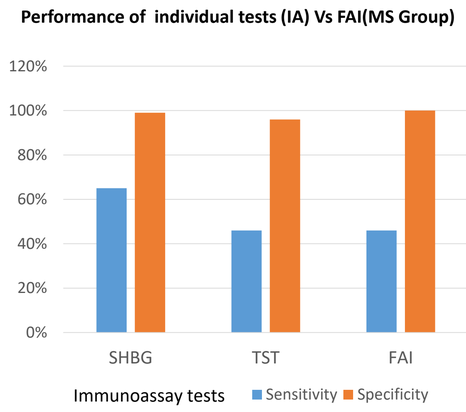
Fig 3. Performance of immunoassay tests vs Mass spectrometry group



The circled area shows the distribution of results classed as Pos within the MS group and Neg within the IA group

Concordance		LC/MS/MS Group Pos FAI	LC/MS/MS Group Neg FAI
immunoassay group	Low SHBG	24	1
	Raised Testo	17	3
	Raised FAI	17	0
	Normal SHBG	13	67
	Normal Testo	20	65
	Normal FAI	20	68

The area outlined in red indicates false-neg results (IA)



Discussion

In contrast to previous reports, immunoassay is now prone to producing lower testosterone results than mass spectrometry in patient samples. When converted to FAI this leads to fewer patients being classified as having 'biochemical evidence of hyperandrogenaemia' vs MS derived FAI results. Although significant improvements are noted in immunoassay, the associated manufacturer's reference data and clinical cohorts may warrant further assessment to ensure biochemical evidence of hyperandrogenaemia as a criteria for identifying PCOS is being more accurately classified. By comparison to the MS group, SHBG alone is a more sensitive indicator of hyperandrogenaemia than either testosterone or FAI derived using Roche immunoassay.

Conclusion

Biochemical evidence of hyperandrogenaemia in the form of FAI is significantly less apparent when derived from immunoassay testosterone vs mass spectrometry, which is likely to have a significant impact on the diagnosis of PCOS.