Female androgen profiles by MS for PCOS patients

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APCCMS 2010, Hong Kong
14 January 2010
### 873 women with increased serum androgens

<table>
<thead>
<tr>
<th>Androgen-secrcting neoplasms</th>
<th>0.2%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical CAH</td>
<td>0.6%</td>
</tr>
<tr>
<td>Non-classical CAH</td>
<td>1.6%</td>
</tr>
<tr>
<td>HAI RAN syndrome</td>
<td>3.1%</td>
</tr>
<tr>
<td>Idiopathic hirsutism</td>
<td>4.7%</td>
</tr>
<tr>
<td>Polycystic ovarian syndrome (hirsutism + normal ovulation)</td>
<td>82% 6.8%</td>
</tr>
</tbody>
</table>

Azziz et al. J Clin Endo & Metab 2004
Polycystic ovarian syndrome (PCOS)

- Diagnostic criteria by Androgen Excess Society (2006)
  - Clinical / Biochemical hyperandrogenism;
  - Ovarian dysfunction / polycystic ovarian morphology; and
  - The exclusion of related or similar disclosures
PCOS

- Affects ~5% of females in the reproductive age

- Associated with
  - Obesity
  - Hirsutism, acne
  - Insulin resistance, diabetes
  - Infertility
Immunoassays for serum female testosterone

Utility, limitations, and pitfalls in measuring testosterone: An Endocrine Society Position Statement.

Solution

- LC tandem MS method for serum total testosterone for female patients
Increased FAI

Increased testosterone

Increased androstenedione

N = 560, (38.3%)  

29%

Oestrogen production in ovary

LH

Cholesterol $\rightarrow$ cAMP $\rightarrow$ Androstenedione $\rightarrow$ Testosterone

Androgenic effects

FSH

Androstenedione $\rightarrow$ Testosterone

Androstenedione $\rightarrow$ Testosterone $\rightarrow$ Estrone $\rightarrow$ Estradiol

Estrogenic effects

Theca cell

Granulosa cell

Aromatization
## Request for 17OHP

<table>
<thead>
<tr>
<th>Cases</th>
<th>Date for high testosterone</th>
<th>Date for 17OHP</th>
<th>Duration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9 May 08</td>
<td>16 Jul 08</td>
<td>68</td>
</tr>
<tr>
<td>2</td>
<td>23 Apr 08</td>
<td>21 May 08</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>10 Jun 08</td>
<td>14 Aug 08</td>
<td>65</td>
</tr>
<tr>
<td>4</td>
<td>9 Jan 08</td>
<td>14 Aug 08</td>
<td>218</td>
</tr>
<tr>
<td>5</td>
<td>22 Nov 06</td>
<td>30 Mar 07</td>
<td>128</td>
</tr>
</tbody>
</table>
Serum female androgens profile service

- Profile
  - Simultaneous measurement of serum androstenedione, testosterone, 17OH-progesterone
  - By specific LC tandem MS method

- Launched August 2008
Waters Quattro Micro
Sample preparation

100 µl serum + 100 µl 0.2 M ZnSO₄
+

100 µl methanol with labeled IS

Centrifugation
**LC method**

- **Gradient mobile phase at 500 µl /min**
  - Solution A: 2 mM ammonium acetate in water + 0.1% formic acid
  - Solution B: 2 mM ammonium acetate in methanol + 0.1% formic acid

- **Column**
  - Waters XBridge C18, 3.5 µm, 2.1 x 30 mm kept at 50 °C

- **Injection to injection: 10 min**
Tandem MS method

<table>
<thead>
<tr>
<th>Compound</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androstenedione</td>
<td>287</td>
</tr>
<tr>
<td>d7-Androstenedione</td>
<td>294</td>
</tr>
<tr>
<td>Testosterone</td>
<td>289</td>
</tr>
<tr>
<td>d2-Testosterone</td>
<td>291</td>
</tr>
<tr>
<td>17OH progesterone</td>
<td>331</td>
</tr>
<tr>
<td>d8-17OH progesterone</td>
<td>339</td>
</tr>
</tbody>
</table>
HPLC Tandem MS procedure

Supernatant

HPLC column

Tandem MS

Waste
Androstenedione
Testosterone
17-OH progesterone
Analytical performance

- Detection limit: 0.5 nmol/l
- Linearity: at least 1000 nmol/l
- Calibration: 0.5 – 50 nmol/l
  - Include a QC sample >1000 nmol/l for 17OHP
Analytical performance

- Between batch precision (n>50)
  - Androstenedione
    - Mean: 2.3 – 14.0 nmol/l
    - CV: 2.7 – 4.1 %
  - Testosterone
    - Mean: 0.9 – 12.8 nmol/l
    - CV: 3.0 – 10.2 %
  - 17OHP:
    - Mean: 1.6 – 769 nmol/l
    - CV: 2.0 – 8.5%
Method correlation for testosterone using 185 PCOS serum samples

LCTMS = 0.75 * E170 + 0.08

$\rho=0.6714$, $p<0.0001$

Passing & Bablok Regression
Method correlation for testosterone using 185 PCOS serum samples
Method correlation of testosterone using 185 PCOS serum samples
Reference intervals

- 180 local healthy pre-menopausal female reference serum samples
- Reference intervals by non-Parametric method:
  - Androstenedione: 1.1 – 6.5 nmol/l
  - Testosterone: < 1.7 nmol/l
  - 17OH progesterone: from UCLH, UK
Validation using PCOS patients

- 185 serum samples from PCOS patients
- Diagnosis (2003 Rotterdam consensus), at least 2 of the following:
  - Oligo-amenorrhoea or chronic anovulation
  - Clinical &/or biochemical hyperandrogenism
  - Ultrasound appearance of polycystic ovaries, after exclusion of other known causes of hyperandrogenaemia
Androstenedione = 22 nmol/l

Testosterone = 4.5 nmol/l

17OH-progesterone = 3.8 nmol/l

PCOS
Diagnosis of hyperandrogenism using E170

- 73 PCOS patients (first visit)
- Testosterone ref range: < 2.9 nmol/l
- 21 had increased testosterone (28.8%)
Diagnosis of hyperandrogenism using the female androgens profile by MS

- 73 patients (first visit)
- Testosterone > 1.7 nmol/l
  - 29 (39.7%)
- Androstenedione > 6.5 nmol/l
  - 51 (69.9%)
- Testosterone + Androstenedione
  - 29 (39.7%)
- 22 patients only had Androstenedione
  - 30.1%
Specific LC tandem MS method for serum testosterone alone improves the detection of androgen excess in PCOS patients

Testosterone + androstenedione further detect androgen excess in PCOS patients – but clinical significance??

The profile improves clinical confidence and turnaround time in diagnosis of female patients with androgen excess
Acknowledgements

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