The role of assay specificity in improving the diagnosis and treatment of endocrine disorders.

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Disclosures: Steroid, 3 thyroid patents issued. Free Vitamin D3 and 1:25 dihydroxy Vitamin D3 patents pending
NIH Intramural research support 2011-2015
NIH CTSA support 2000-2011 (Children’s National and Georgetown University)

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To prescribe the right treatment, it is essential to have the correct diagnosis.

Correct diagnosis is dependent on reliable and accurate quantitation of disease markers

Disease markers need to correlate with the clinical picture.

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From colorimetric to HPLC amperometric detection for catecholamines / metabolites. Gil Hill, Bill Purdy McGill University

These assays markedly improved diagnosis and management of pheochromocytoma and neuroblastoma patients.
Message: Lack of specificity in measurement changes both the means and widens the Gaussian distributions for the normal and diseased populations


Marks the beginning of lessons in specificity dating back to 1980s.

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THYROID ISSUES

Why should we be interested in thyroid disorders?

Total prevalence of hypothyroidism is 5.7% (19 million in USA).
Total prevalence of hyperthyroidism is 3.1% (10 million in USA).
[European Journal of Endocrinology (2000) 143 639-647]

Endocrinologists constantly complaining about lack of correlation of free thyroid tests with TSH. CAP PT program shows major differences between different immunoassays.

Evidence is mounting that a substantial percentage of patients with hypothyroidism require T4 plus T3 replacement therapy. “Many hypothyroid patients are not tolerating and dissatisfied with LT4 treatment (palpitation, anxiety, insomnia, hypothyroid symptoms).” Correct diagnosis depends on reliable measurement of T4, T3, FT4, FT3 and TSH.

Suggested mechanisms of action

T4 pre-hormone…..D1 (plasma membrane)
D2 (Endoplasmic reticulum)

T3 active combines with TRs in nucleus triggering biological effects

D3 plasma and nuclear membrane deactivates T3 to T2 and T4 to rT3

FT4 vs Log TSH

Spencer at. al. J Clin Endocrinol Metab 1990

ABBOTT ARCHITECT ci8200: Correlation of FT4 vs. log TSH: Males

$y = -0.0066x + 1.013, r = 0.010, n = 2654.$

NIH Study 2010 n=109 UF MSMS

Inverse log-linear relationship: LC-MS/MS-FT$_4$ and Log TSH

$R = 0.84$ (95% CI 0.77 to 0.88)


FT4 vs Log TSH

<table>
<thead>
<tr>
<th>IA Studies</th>
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<tbody>
<tr>
<td>Platform</td>
</tr>
<tr>
<td>Siemens RxL Dimension</td>
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<tr>
<td></td>
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<tr>
<td>Abbott Architect c8200</td>
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<tr>
<td>Siemens Immulite 2000</td>
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<tr>
<td>Beckman Coulter Access DXI 800 Unicel</td>
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<tr>
<td>Roche Modular E170</td>
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<td>Siemens ADVIA Centaur</td>
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<tr>
<th>Mass Spectrometry Studies</th>
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<td>Studies by</td>
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**POST THYROIDECTOMY TSH vs T3**


**Georgetown University in and out-patient study**

IA Siemens Immulite vs MS/MS [9 vs 48 below 2.5th percentile. BIAS T intercept is 34 T]

Soukhova N, Soldin OP, Soldin SJ. CCA 2004; 343: 185-60


**Roche 6000 T3 IA/MS comparisons at various concentration ranges**

MS classifies 69% as below 2.5th percentile. IA classifies 35% as below the 2.5th percentile.

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Georgetown in-patient study in non-thyroidal diseases.

Summary:
- Patients did not have underlying/overt thyroid disease
- 48% of the T3’s were below the 2.5th percentile when measured by MSMS
- 11% of them were below 2.5th percentile when measured by IA (Siemens Vista)
- In-patients are on approximately 6-8 drugs.

QUESTION:
- Are these multiple drug regimens affecting the deiodinases resulting in low T3’s

See Abdalla SM and Bianco AC, “Defending plasma T3 is a biological priority” Clinical Endocrinology 2014;0:1-9

70 y old woman with Hashimoto’s thyroiditis and problems converting T4 to T3:

IA results for T4, T3, FT4, FT3, TSH normal. Physicians refuse to change dose regimen as all tests normal. All FDA approved tests-poor correlation with TSH or log TSH. Patient complains of feeling sluggish and unwell.

Mass Spectrometry results—good correlations with TSH

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Iodothyronamine</td>
<td>&lt;5 pg/mL</td>
<td>(&lt;5 pg/mL)</td>
</tr>
<tr>
<td>T2</td>
<td>4.6 pg/mL</td>
<td>(7.8-22.2 pg/mL)</td>
</tr>
<tr>
<td>T3</td>
<td>74 ng/dL</td>
<td>(80-166 ng/dL)</td>
</tr>
<tr>
<td>rT3</td>
<td>9.4 ng/dL</td>
<td>(9.2-22.8 ng/dL)</td>
</tr>
<tr>
<td>T4</td>
<td>10.0 ug/dL</td>
<td>(4.9-10.6 ug/dL)</td>
</tr>
<tr>
<td>FT4</td>
<td>1.9 ng/dL</td>
<td>(1.3-2.4 ng/dL)</td>
</tr>
<tr>
<td>FT3</td>
<td>2.5 pg/mL</td>
<td>(1.8-6.2 pg/mL)</td>
</tr>
</tbody>
</table>

Patient added T3 to dosing regimen
Her FT3 is now fine at 3.3 pg/mL (1-6) and patient better

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50 y female with hypothyroidism

Complains of tiredness and feeling sluggish.

IA: FT4 1.0 (0.8-1.5); T3 91 (90-215); TSH 1.28 (0.4-4.0)

MSMS: FT4 2.1 ng/dL (1.3-2.4); FT3 1.9 pg/mL (1.5-6.0); T3 79 ng/dL (80-187); T4 7.1 ug/dL (4.9-10.5)

Added 12.5 ug T3 bid.

Patient reports feeling well.

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68 YEAR OLD WOMAN WITH HYPOTHYROIDISM FEELING LETHARGIC

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT4</td>
<td>1.2 ng/dL</td>
<td>(0.8-2.0)</td>
</tr>
<tr>
<td>FT3</td>
<td>1.5 pg/mL</td>
<td>(1.5-6.0)</td>
</tr>
<tr>
<td>FT3</td>
<td>64 ng/dL</td>
<td>(80-187)</td>
</tr>
<tr>
<td>rT3</td>
<td>8.7 ng/dL</td>
<td>(9-21)</td>
</tr>
<tr>
<td>T4</td>
<td>7.2 ug/dL</td>
<td>(4.9-10.5)</td>
</tr>
</tbody>
</table>

Heterozygous for human D2 Thr92Ala polymorphism (33-60% of population)

Treatment with T3 normalized MSMS measurements and improved clinical condition. Cholesterol normalized, 220 to 160 mg/dL.


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Binding protein effects on FT4/FT3 by IA: 30 year old male AIDS-related Kaposi sarcoma

- Immunoassay results
  - FT4 0.66 ng/dL (0.8-1.5) L Euthyroid patient, IA results do not correlate with clinical condition.
  - FT3 1.41 pg/mL (1.80-4.20) L
  - TSH is 3.52 uIU/mL (0.36-4.0) N
  - T3 is 42 ng/dL (90-215) L
  - T4 is 4.3 ug/dL (13-39) L
  - TBG is 32 ng/mL (1.6-60) N
- Mass spec results
  - FT4 1.8 ng/dL (1.4-3.2) N Results correlate with clinical symptoms
  - FT3 1.7 pg/mL (1.5-6.0) N
  - T4 2.4 ug/dL (4.9-10.2) L
  - T3 25.2 ng/dL (83-168) L
  - rT3 10.7 ng/dL (9-23) N

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56 YEAR OLD CAUCASIAN FEMALE WITH 3 YEAR HISTORY OF HYPOTHYROIDISM

She presented with lethargy, loss of energy and struggling to lose weight. BMI 29.9 kg/m². Patient was treated with Synthroid only and still feels lethargic, is also constipated.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>MSMS</th>
<th>Immunoassay</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4 ug/dL</td>
<td>13.4(5.1-11.3)</td>
<td>11.8 (4.5-11.7)</td>
</tr>
<tr>
<td>FT4 ng/dL</td>
<td>1.9(1.3-2.4)</td>
<td>1.6 (0.9-1.7)</td>
</tr>
<tr>
<td>TSH uIU/mL</td>
<td>2.65 (0.27-4.2) Normal</td>
<td></td>
</tr>
<tr>
<td>T3 ng/dL</td>
<td>82 (80-187) [3rd %]</td>
<td>114 (80-200)[25th%]</td>
</tr>
<tr>
<td>FT3 pg/mL</td>
<td>2.1 (1.5-6.2)[4th%]</td>
<td>2.6 (2.0-4.4)[20th%]</td>
</tr>
<tr>
<td>Cholesterol mg/dL</td>
<td>219(&lt;200)</td>
<td></td>
</tr>
<tr>
<td>TBG normal, ATA and ATG below detection limit</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Now started on additional dosing BID with 12.5 ug T3.

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Case report continued

The low MS FT3 and TT3 may explain the unresolved symptoms of hypothyroidism even with the now normalized FT4 and TSH. Patient was started on 12.5 ug bid T3. After 10 days of treatment all her symptoms of hypothyroidism were alleviated.

Also cholesterol dropped from 219 to 190 mg/dL.

Her 10 day post T3 treatment LC-MSMS results for FT3 and TT3 at 8am (pre-dose ) were 3.8pg/mL[1.5-6.2] and 129 ng/dL[80-187] and 2h post dose were 4.6 pg/mL and 145 ng/dL respectively.

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“Perhaps 20% of hypothyroid patients treated with T4 alone continue to complain of symptoms suggesting thyroid hormone deficiency? deiodinase polymorphisms. These patients may benefit from T3/T4 combination therapy!!”

When patients complain and results do not correlate with the clinical picture there is usually a good reason.

What (wo)men do to (wo)men

“Do no harm”,
Thomas Dunne

FDA’s contribution to diagnostic errors

- FDA has approved all the “problem” tests (IA’s for FT4, FT3, T3) without assessing whether any of them are “clinically useful” (good correlation to: 1. log TSH and 2. clinical condition).

- One could argue that the current system provides clinicians with the wrong result thereby contributing actively to both incorrect diagnoses and subsequent treatment.

- In the low range, IA’s give false normal results in 65% of FT4’s and 50% of FT3’s and T3’s. This affects 4-6 million hypothyroid people in the USA alone. These tests therefore will account for many wrong diagnoses resulting in incorrect or no treatment with T3 when T3 treatment is warranted.
Summary and conclusions for thyroid hormone testing

T4 testing by immunoassay appears to be reliable.

T3 testing by immunoassay is suboptimal at low concentrations, during pregnancy, and in many in-patients.

FT4/FT3 and TT3 tests need to correlate with log TSH. The FDA has a role to play when they license IA for FT4, FT3, and TT3.

We recommend that UF-MSMS be employed to measure FT4/FT3 for all specimens in which the TSH >95%-tile or <5%-tile. We also recommend that IA T3’s below the 10th percentile be repeated by LCMSMS.

T4, T3, FT4, FT3 tests by LCMSMS are available at commercial laboratories.

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Lack of Specificity of FDA Approved Immunoassays.
Data from CAP PT Program Y-A Survey 2010

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Low</th>
<th>High</th>
<th>H/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (ng/dL)</td>
<td>83</td>
<td>213</td>
<td>(2.6)</td>
</tr>
<tr>
<td></td>
<td>715</td>
<td>1309</td>
<td>(1.8)</td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>38</td>
<td>366</td>
<td>(9.6)</td>
</tr>
<tr>
<td></td>
<td>274</td>
<td>1140</td>
<td>(4.2)</td>
</tr>
<tr>
<td>Progesterone (ng/mL)</td>
<td>4.6</td>
<td>6.3</td>
<td>(1.4)</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>12.5</td>
<td>(1.7)</td>
</tr>
<tr>
<td>17OH P (ng/dL)</td>
<td>765</td>
<td>2121</td>
<td>(2.8)</td>
</tr>
</tbody>
</table>
Tandem Mass Spectrometry.
Data from CAP PT Program Y-A Survey 2010

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Low</th>
<th>High</th>
<th>H/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (ng/dL) n=13</td>
<td>71</td>
<td>125</td>
<td>(1.7)</td>
</tr>
<tr>
<td></td>
<td>635</td>
<td>959</td>
<td>(1.5)</td>
</tr>
<tr>
<td>17OH P (ng/dL) n=11</td>
<td>821</td>
<td>1289</td>
<td>(1.5)</td>
</tr>
<tr>
<td>Estradiol (pg/mL) n=4 NYS PT</td>
<td>142</td>
<td>184</td>
<td>(1.3)</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>152</td>
<td>(3.4)</td>
</tr>
<tr>
<td>Progesterone (ng/mL) n=4 NYS PT</td>
<td>3.9</td>
<td>4.8</td>
<td>(1.2)</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>67</td>
<td>(1.4)</td>
</tr>
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SINGLE ANALYTE vs PROFILE

- The case of large commercial reference laboratories
- The majority of teaching hospitals have less than 1,000 beds and a single tandem MSMS performing steroid profiles would provide physicians to access >1 steroid in the panel.
- Panel testing provides the clinician with more information

3rd Generation Steroid Profile Assay by LCMSMS

Recent new steroid profile applications

CAH
PCOS (most common gynecological endocrinopathy 7-14% of women)
Marathon running
Adrenal insufficiency (0.6%)
Diurnal effects on steroid concentrations
Neurosteroids

Case Study CAH: Steroid Panel

- 2 day infant, normal penis, no palpable testes
- 17 OH Prog >20,000 ng/dL (<100 ng/dL)
- 11 Deoxycortisol 1.2 ug/dL (<0.15)
- Testosterone 441 ng/dL (<10 ng/dL)
- Androstenedione >11,900 ng/dL (<50 ng/dL)
- Progesterone 3.31 ng/mL (<0.33 ng/mL)
- DHEAS 15 ug/dL (<1.25 ug/dL)
- CAH with 21 OH-ase deficiency (1 in 15 thousand births) Low cortisol (3.8 ug/dL)
- Karyotype on peripheral blood 46 XX
- Early detection leads to early treatment (glucocorticoid replacement) and avoids salt wasting crises.
<table>
<thead>
<tr>
<th>DRUG TREATMENT OF CAH</th>
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</thead>
<tbody>
<tr>
<td>DRUG</td>
</tr>
<tr>
<td>Hydrocortisone/cortisone</td>
</tr>
<tr>
<td>Prednisolone</td>
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<tr>
<td>Dexamethasone</td>
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</tbody>
</table>

Hydrocortisone preferred medication for children with CAH
If a salt-retaining hormone is needed, use fludrocortisone

Hormonal Circadian Rhythms in Patients with Congenital Adrenal Hyperplasia:

The treatment goal in congenital adrenal hyperplasia (CAH) is to replace glucocorticoids while avoiding androgen excess and iatrogenic Cushing’s syndrome.


Continuous Subcutaneous Hydrocortisone Infusion (CSHI)

Background: CAH treatment focuses on replacement of cortisol and aldosterone and prevention of ACTH – driven androgen excess. This approach is frequently inadequate.

5 patients with difficult to treat (7am 17-OHP>1200 ng/dL and androstenedione >210 ng/dL) classic CAH due to 21-hydroxylase deficiency.

Results: At 6 months all but 1 patient had 7am 17-OHPs 2-6 fold lower than baseline values.

Conclusion: CSHI is a safe and well-tolerated therapy.

Paper submitted to JCEM
Summary PCOS Study
30 PCOS, 30 age matched controls
DHEA,DHEAS,Androstenedione,
Testosterone and Aldosterone are higher in patients with PCOS.
5/30 PCOS patients had very elevated aldosterones (>550 pg/mL) some of which were due to an adrenal tumor.
Conclusion: results emphasize the power of profile monitoring

Marathon runner study (n=51, distance 52 Km)

- Pre vs post race steroid profiles
- Aldosterone 6.1 to 19.7 ng/dL p<0.001
- Corticosterone 226 to 3491 ng/dL p<0.001
- 11-Deoxycortisol 0.03 to 0.54 ug/dL p<0.001
- Cortisol 10 to 33 ug/dL p<0.001
- DHEA,DHEAS and Androstenedione all showed increases p<0.001
Box and whiskers plots of am versus pm values for males and females for the steroids: a. 11-DOC, b. Corticosterone, c. 17-OHP, d. Cortisol, e. Cortisone, f. Androstenedione, g. Testosterone values for males only. (Mean is indicated by small square markers and median by bar in box, p<0.05 is considered statistically significant)

Stolze, Gounden, Gu, Soldin Clin Chem 2015:61;556-58

Summary of Diurnal rhythms for steroids

- Diurnal effects are very significant for most steroids studied
- This means that it is necessary to standardize collection times and have reference intervals for 8am, 8pm and midnight

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Summary of Adrenal Insufficiency Study

- Change the textbooks! Cortisol is not the best marker to measure after ACTH stimulation.

- The combination of 11 DOC, Cortisol, DHEA, and Aldosterone optimizes correct classification of primary vs secondary adrenal insufficiency. Aldosterone concentrations <60 pg/mL at 30 min = primary AI.

Holst JP, Soldin SJ et al. Steroids 2007;72;71-84

Neurosteroids

FACT-F: cancer fatigue scores. All subjects are men with prostate cancer who have received radiation therapy. Fatigue and neurosteroid levels were measured at one year after radiation therapy.
Studies on free vitamin D3 in our laboratory


Why do we need to assess free vitamin D3 levels?

There is a poor correlation between total vitamin D3 and PTH

Some areas of clinical interest for free vitamin D3 include:

Depressive illness, Seizure disorders, Oncology, Bone diseases, Alzheimer’s disease, Schizophrenia, Autism, Parkinson’s disease, Multiple Sclerosis, Pregnancy

How to measure free vitamin D3

Measurement of many free steroid and thyroid concentrations is easy. Why did we fail for so long to develop a method for measurement of free Vitamin D3?

Vitamin D3 binds to its binding protein in serum/plasma. Ultrafiltration results in a protein free ultrafiltrate. Vitamin D3 in this ultra-filtrate sticks to both glass and plastic. However, if filtered into an organic solvent it remains in solution and can be quantified.
I love talking about nothing. It is the only thing I know anything about.

Oscar Wilde

We are all amateurs……..we don’t live long enough to be anything else

Charlie Chaplin, Limelight

Don’t let schooling interfere with your education. Grant and/or Mark Twain

Final comment on fate of creative people:

“It is the finest blades that are most easily blunted, bent or broken”