

Testosterone measurement – Which test and when?

Martin E Gleave MD, FRCSC, FACS

British Columbia Leadership Chair
Distinguished Professor and Chair,
Department of Urologic Sciences, UBC
Director, Vancouver Prostate Centre

Synthesis of Testosterone

Nobel Prize, Chemistry, 1939



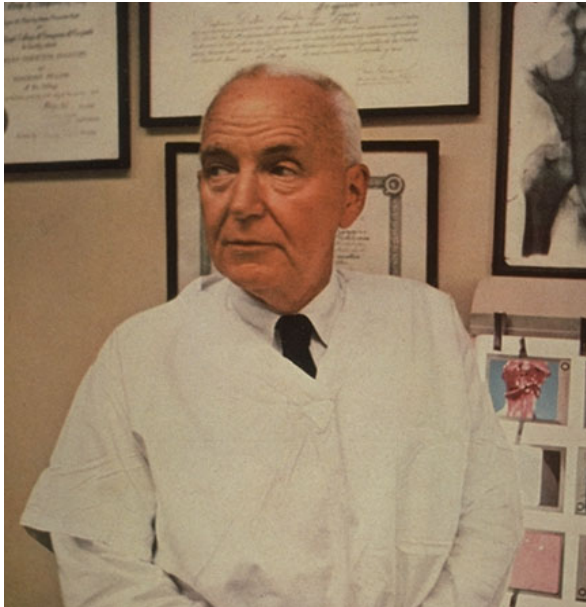
Adolf Butenandt
(Germany)



Leopold Ruzicka
(Switzerland)

“Until recently, very little was known about the sex hormones.”

Nobel Prize in Physiology or Medicine 1966: for Discoveries Concerning Hormonal Treatment of Prostatic Cancer



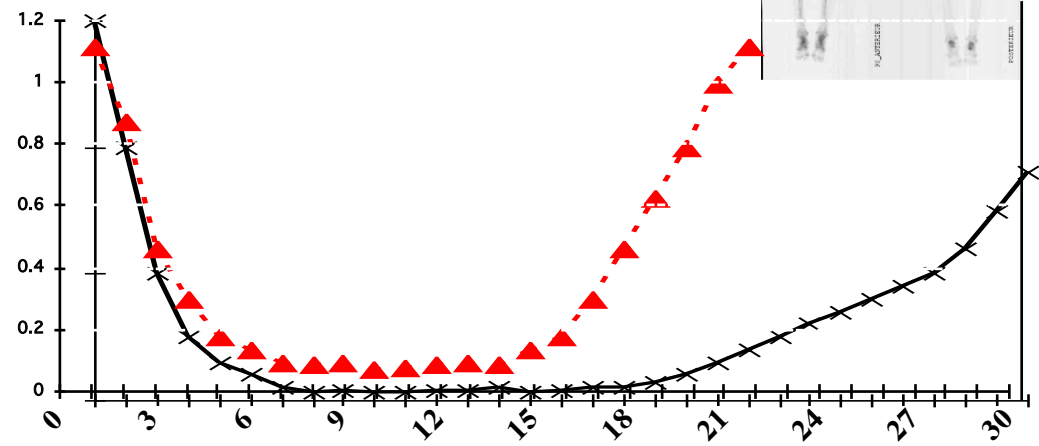
Nobel Laureate, 1966

Charles Huggins

Urologic-scientist at U of Chicago



ADT



Peptide Hormones of Brain (LHRH)

Nobel Prize, Physiol/Med, 1977



Roger Guillemin
(1924 -)

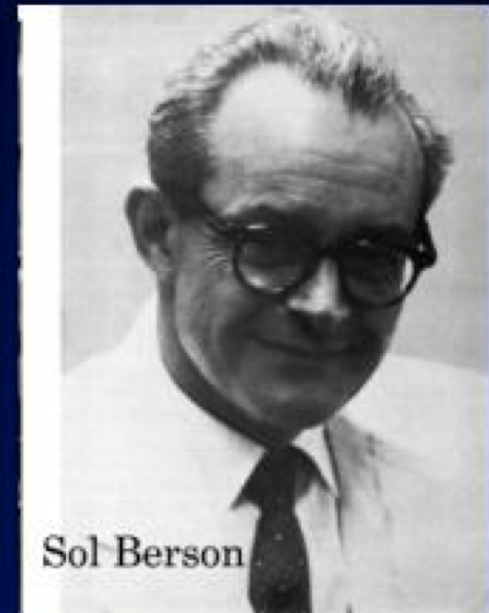


Andrew Schally
(1926 -)

*"They have uncovered a substantial part
of the link between body and soul."*

Radioimmunoassay (RIA)

Nobel Prize, Physiol/Med, 1977



“Before Yalow, these hormones could not be determined quantitatively in the blood.”

T Measurement

Why important?

- androgens drive AR activity
- maintenance of castrate T required in mCRPC guidelines
 - Key for intermittent and continuous ADT
 - By consensus, T <50 ng/dL (1.7nM) defined as castrate
 - lowest levels of serum androgens key therapeutic goal (best achieved by ADT + ABI combinations)
- serum T (and perhaps other SA's) prognostic for time to CRPC and potentially predictive to ARPI

1.7 nmol/L = 50 ng/dL
0.7 nmol/L = 20 ng/dL
0.3 nmol/L = 8.6 ng/dL

Some Patients on LHRH Agonist Do Not Achieve Castration T Levels

- Depends on benchmark (surgical castrate T levels ~ 0.7 nM¹)
 - At 1.7 nmol/L:¹⁻⁴
 - 88% to 97% will achieve castration
 - At 0.7 nmol/L:^{1,2,4,5}
 - 63% to 87% will achieve castration
- LHRHa + ABI – usually < 0.3 nM
- Time to CRPC on LHRHa has been linked to serum T, including PR-7⁶ and ICELAND⁷

0.3 nmol/L = 8.6 ng/dL
0.7 nmol/L = 20 ng/dL
1.7 nmol/L = 50 ng/dL

*Using varied immunoassays

1. Oefelein MG, et al. J Urol 2000;164:726-9
2. Morote J, et al. Urol Int 2006;77:135–8;
3. Wechsel HW, et al. Eur Urol 1996;30(Suppl 1):7-14;
4. McLeod D, et al. Urology 2001;58:756-61;
5. Kawakami J, et al. Can Urol Assoc J 2013;7:E226-30
6. Klotz L, et al. J Clin Oncol 2015;33:1151-6
7. Tombal B, et al. Ann Oncol J Urol 2017;198:1054-60

Serum Androgens As Prognostic Biomarkers in Castration-Resistant Prostate Cancer: Results From an Analysis of a Randomized Phase III Trial

Charles J. Ryan, Arturo Molina, Jinhui Li, Thian Kheoh, Eric J. Small, Christopher M. Haqq, Russell P. Grant, Johann S. de Bono, and Howard I. Scher

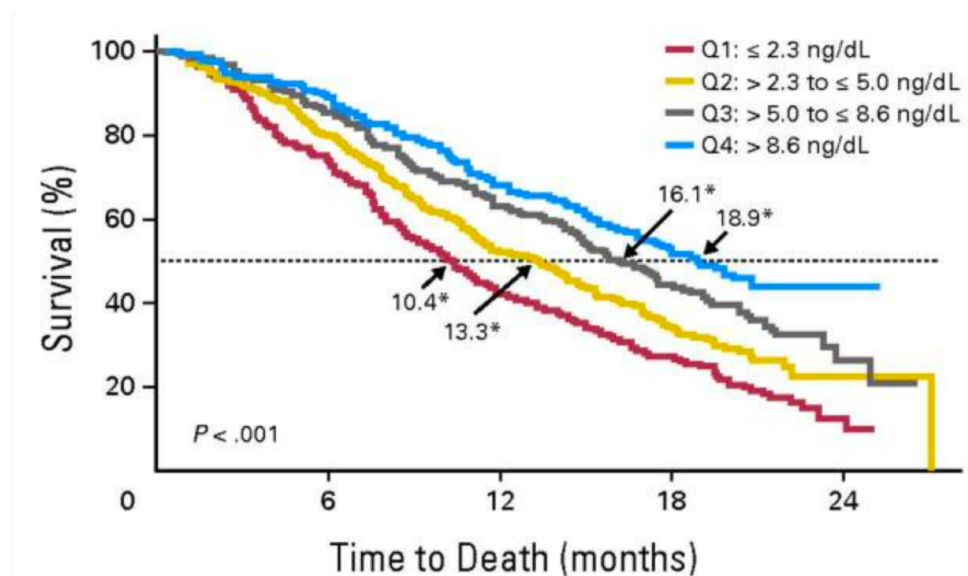
Charles J. Ryan and Eric J. Small,
University of California, San Francisco,
San Francisco; Arturo Molina, Thian

A B S T R A C T

J Clin Oncol 31:2791-2798. © 2013

Serum T levels in mCRPC, measured by LC-MS/MS, are prognostic for OS in Cou-301 trial

- Longer OS for patients with SA above median ($P < .0001$).



T Measurement

Why (is it an issue in PCA)

- quantification of T (and SA) challenging at low levels in men on ADT
 - limits of quantification
 - less of an issue with ADT + ABI in mCSPC
- Other factors beyond quantification
 - ligand bioavailability (eg. SHBG, intratumoral levels)
 - consider relevant ligands in castrate-sensitive (AR^{wt}) and resistant (AR^{amp} , AR^{mut}) states

T Measurement

Which Assay

- Historically serum T measured with solvent extraction followed by RIA or GC-MS
- Automated immunoassays for total T developed for high-throughput, clinical laboratory environment
- IA's inaccurate at low T concentrations due to endogenous and exogenous interferences and issues with calibration at low analyte concentrations
 - Lower limits of quantification (sensitivity threshold) near castrate levels

LC-MS vs ELISA Steroid Analysis

ELISA

Pros:

- Good sensitivity
- Easy to do, quick
- Often no extraction needed
(Ab/matrix dependent)
- Relatively inexpensive equipment

Cons:

- Potential for cross-reactivity/bias
- Narrow dynamic range
- Competitive assay – non linear cal
- Ongoing reagent costs
- Single steroid at a time

Wide selection of ELISA kits – performance varies

LC-MS

Pros:

- High selectivity/low bias
- High levels of multiplexing possible
- Good sensitivity with most steroids *
- Wide dynamic range
- Low reagent costs

Cons:

- Expensive instrumentation needed
- Technical expertise more critical
- More sample prep required
(SPE or liq/liq extraction; derivatizing)
- More expensive than IA

Potential for inaccurate results due to calibration errors is shared by both platforms

LC-MS vs ELISA Steroid Analysis

- 10 T immunoassays compared to a GC-MS assay¹
- IA's gave results up to 5-fold higher in females (“clinically useless”) than the results by GC-MS

1. J. Taieb, et al Clin. Chem. 49 (8) (2003) 1381–1395.

2. W. Rosner, et al J. Clin. Endocrinol. Metab. 92 (2) (2007) 405–413

Roche Elecsys Testosterone II Assay

Precision claims at level of:

0.316 nM the SD= 0.049 and CV = 14.8%
2.42 nM the SD=0.097 and CV=4.1%.

Method comparison vs. ID-GC/MS shows excellent correlation down to 0.173 and up to 60.0 nM

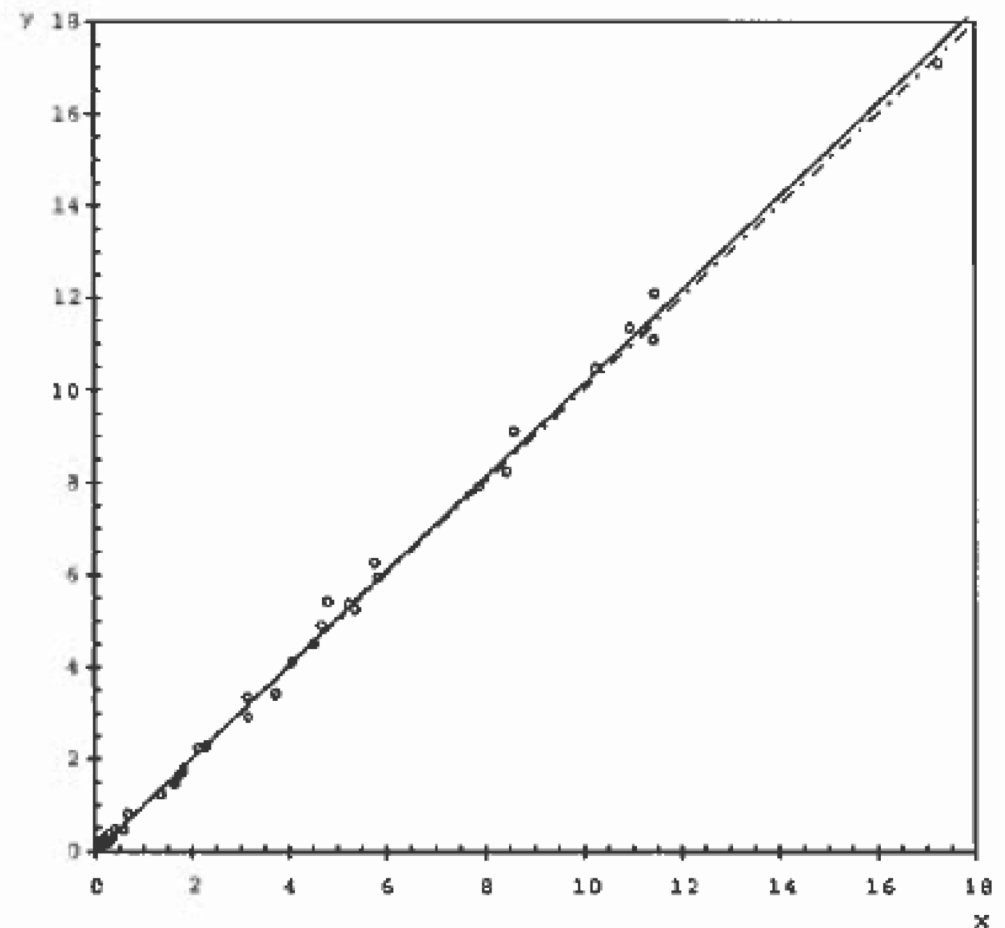
Why is there still variation at low levels in clinical samples?

- **bias (specificity): cross-reactivity with other steroids**

Method comparison

a) A method comparison of the Elecsys Testosterone II assay (y) with the ID-GC/MS method (x) using 39 serum samples gave the following correlations (ng/mL):

Samples from males and females (n = 39):



x: ID-GC/MS (ng/mL)

y: Elecsys Testosterone II assay (ng/mL)

• Points

--- x = y

— Passing/Bablok

--- Linear regression

Passing/Bablok¹³

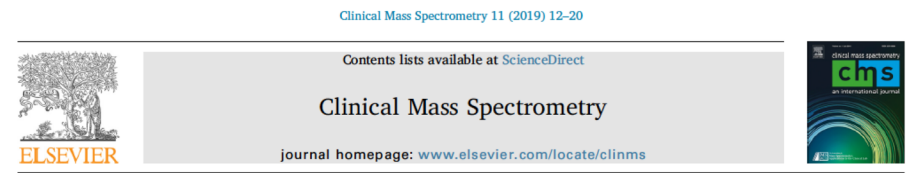
Linear regression

LC-MS/MS Measurement of T

- LC-MS/MS is most sensitive, specific, precise and accurate measurement of T
- BUT still subject to errors if methods not properly developed, validated, implemented
- Overall imprecision of measurements in 7 different LC-MS/MS assays was <15% at T >1.53 nmol/L and <34% at 0.3 nmol/L

H.W. Vesperet al. Steroids 74 (6) (2009) 498–503

- Excellent agreement between 4 independently developed LC-MS/MS assays demonstrates that harmonization using standard reference material is attainable
- Can quantify other SA's in one assay



Comparison of four clinically validated testosterone LC-MS/MS assays:
Harmonization is an attainable goal

Deborah French^{a,*}, Julia Drees^b, Judith A. Stone^c, Daniel T. Holmes^{d,e}, J. Grace van der Gugten^d

^a Department of Laboratory Medicine, University of California San Francisco, San Francisco, CA 94107, United States

^b Kaiser Permanente Northern California Regional Laboratory, Richmond, CA, United States

^c University of California San Diego Health Center for Advanced Laboratory Medicine, San Diego, CA, United States

^d Department of Pathology and Laboratory Medicine, St Paul's Hospital, Vancouver, British Columbia, Canada

^e Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada

LC-MS Multiplexed Assay



VANCOUVER
PROSTATE CENTRE
A UBC & VGH Centre of Excellence

Instrumentation - UPLC/Quattro Premier

	~ ng/ml assay sensitivity				HA fmol on column
	non deriv	HA	FMP	Dansyl	
steroid					
DHEA	0.1	0.03	0.01		1.6
Androstenedione	0.1	0.03	-		1.6
4-Pregnen-17-ol-3,20-dione	0.1	0.03	-		1.4
Testosterone	0.1	0.01	0.02		0.5
* DHT	0.5	0.1	0.01		5.2
Androsterone	1	0.01	0.02		0.5
** 5 α -Pregnan-3,17-diol-20-one	1	0.03	0.02		1.3
pregnenolone	10	0.1	0.05		4.7
progesterone	0.1	0.02	-		1.0
5 α -pregnan-3,20-dione	10	0.05	-		2.4
estradiol	10	-	0.02	0.02	
adrostenediol	10	-	0.01		
androstenediol	2	-	0.02		
adrostenediol-gluc	0.1				3.2

Accuracy and precision within FDA

Bioanalytical guidelines

(15% ; 20% @ LOQ)

Recoveries 80-100%

RESEARCH

Discordance between testosterone measurement methods in castrated prostate cancer patients

Mélanie Rouleau^{1,*}, Francis Lemire^{1,*}, Michel Déry², Benoît Thériault¹, Gabriel Dubois¹, Yves Fradet¹, Paul Toren¹, Chantal Guillemette³, Louis Lacombe¹, Laurence Klotz⁴, Fred Saad⁵, Dominique Guérette² and Frédéric Pouliot¹

¹Division of Urology, Department of Surgery and Cancer Research Center, Centre Hospitalier Universitaire (CHU) de Québec-Université Laval, Québec, Québec, Canada

²Biochemistry Service, Medical Laboratory Department, CHU de Québec-Université Laval, Québec, Québec, Canada

³Pharmacy Faculty, Université Laval and CHU de Québec-Université Laval, Québec, Québec, Canada

⁴Sunnybrook Health Sciences Centre, University of Toronto, Toronto, Ontario, Canada

⁵Centre Hospitalier de l'Université de Montréal, Montréal, Québec, Canada

- Retrospective evaluation of 435 serum samples from castrated PCA patients.
 - Serum T levels measured in same sample by LC-MS and immunoassay
 - Mean T levels higher with IA than with MS (0.672 vs 0.461 nM; $P < 0.0001$)
 - Half of samples with $T \geq 0.7$ nM by IA were < 0.7 nM using MS
 - “T breakthrough” > 0.7 nM was higher with IA than MS (22.1% 13.1%) $P < 0.0001$.
 - Only 3% of samples with $T < 0.7$ nM by IA were ≥ 0.7 nM using MS
- Suboptimal T levels in castrated patients should be confirmed by either MS or a validated immunoassay and interpreted with caution before any changes are made to treatment

CONSENSUS STATEMENT

Testosterone suppression in the treatment of recurrent or metastatic prostate cancer — A Canadian consensus statement

Laurence Klotz, MD, FRCSC¹; Bobby Shayegan, MD, FRCSC²; Chantal Guillemette, PhD³; Loretta L. Collins, PhD⁴; Geoffrey Gotto, MD, MPH, FRCSC⁵; Dominique Guérette, PhD, CSPQ, FCACB³; Marie-Paule Jammal, MD, FRCSC⁶; Tom Pickles, MD, FRCPC⁷; Patrick O. Richard, MD, MSc, FRCSC⁸; Fred Saad, MD, FRCSC⁹

Can Urol Assoc J. 2018;12:30-37

In men receiving ADT for castrate sensitive prostate cancer:

- 1a.** There appears to be a clinical benefit associated with achieving a serum testosterone level of ≤ 0.7 nmol/l (Category 2A)
- 1b.** Testosterone suppression to ≤ 0.7 nmol/l is a reasonable clinical goal (Category 2A)

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Can Urol Assoc J. 2018;12:30-37

In men receiving ADT for prostate cancer:

2a. Prescribers of ADT should perform regular monitoring of T and PSA levels throughout the first year of treatment (Category 2A)

2b.1. Immunoassays may not be sufficiently specific, sensitive, or accurate for low levels of serum T unless the method is validated against MS (Category 2A).

Validated LC-MS/MS methods are the gold standard for T assays at low castrate concentrations (≤ 0.7 nmol/l; Category 2A)

Conclusions: T Measurement Which Test and When

- Lower T levels correlate with better freedom from CRPC
 - Breakthrough T levels > 1.7 nmol/L higher rates of failure
 - Rarely seen with LHRHa/ABI combinations
- Prescribers of LHRHa should periodically assess T levels to ensure castrate levels
 - Measure PSA and T every 3 to 6 months
 - Confirm lab assay is accurate to low levels of T
 - IA's over-estimate low levels of T in men on ADT (more of an issue if underestimates and misses a pt who is not castrate)
 - consider validation (LC-MS if available), change therapy if $T > 0.7$ nM
- Role of T and other SAs as prognostic and predictive biomarkers with ARPI in mCRPC requires more study
 - Genomic and metabolic alterations support AR-reactivation