MISURA DEGLI STEROIDI NEI LIQUIDI BIOLOGICI: APPROPRIATEZZA, LIMITI E INSIDIE DIAGNOSTICHE

ROMA, 10 NOVEMBRE 2019

Misura degli steroidi: immunometria e/o spettrometria di massa?

Renato Tozzoli

Coordinatore Gruppo di Studio SIPMeL Endocrinologia e Malattie del metabolismo

Conflitti di interesse

Ai sensi dell'art. 4.5 su "Docenti e moderatori dell'evento", pag. 8 del Manuale Nazionale di Accreditamento per l'erogazione di eventi ECM del 06/12/2018, dichiaro che negli ultimi 2 anni **non** ho avuto rapporti diretti di finanziamento con soggetti portatori di interessi commerciali in campo sanitario.

Misura degli steroidi: immunometria e/o spettrometria di massa?

- Le ragioni del dibattito/conflitto tra metodi
- Le caratteristiche dei metodi
- La pratica corrente di laboratorio
- Gli ormoni di interesse (attuale) per LC-MS/MS
  - Cortisolo urinario e salivare
  - 17OH-progesterone sierico
  - Testosterone sierico

LC-MS/MS e IMA: sostituzione o integrazione?

Requirement for Mass Spectrometry Sex Steroid Assays in the *Journal of Clinical Endocrinology and Metabolism*

D. J. Handelsman and L. Wartofsky

ANZAC Research Institute (D.J.H.), Concord Hospital, University of Sydney, Sydney, NSW 2139, Australia; and Department of Medicine, Washington Hospital Center (L.W.), Washington, DC 20010

J Clin Endocrinol Metab, October 2013, 98(10):3971–3973

Effective January 1, 2015, manuscripts reporting sex steroid assays as important end-points must use MS-based assays including reporting and citing their metods with sufficient details …

ENDOCRINE SOCIETY

February 12, 2014

Important Notice on JCEM Mass Spectrometry Sex Steroids Assays Requirement

In the October 2013 issue of the *Journal of Clinical Endocrinology and Metabolism* (JCEM), a policy requiring mass spectrometry sex steroid assays was published. This new JCEM policy raises important and valuable scientific issues that require broader consideration. In order to properly address the complexity of the issues, the requirement for using mass spectrometry sex steroid assays, which was scheduled to go into effect on January 1, 2015, is suspended pending further scientific review.

A task force of experts is being convened to review scientific policies regarding the reporting of sex hormone measurements in the Society's journals. Once this review is completed, any new policies and/or clarifications will be announced at that time. In instituting a time line for any policy changes, we will be sensitive to the needs of clinical investigators who are conducting and planning studies. We anticipate that the task force will complete their scientific review by June 30, 2014.

Scientific excellence and the concerns of our readers and authors are of the utmost importance to the Society. Please do not hesitate to share your feedback with either of us.

Sincerely,

Teresa K. Woodruff, PhD
President

Margaret Shupnik, PhD
Chair, Publications Core Committee

In order to properly address the complexity of the issues, the requirement for using mass spectrometry sex steroid assays, which was scheduled to go into effect on January 1, 2015, is suspended pending further scientific review.

Caratteristiche degli IMA

60 anni di storia per peptidi (insulina): Yalow e Berson, 1959

50 anni di storia per steroidi (estradiolo): Abraham, 1969

Semplificazioni della misura: dosaggio diretto

Inaccuratezza metodo-specifica

Aspecificità analitica

Bassa sensibilità analitica

Caratteristiche degli IMA

- Elevato throughput
- Basso costo
- Automazione
- La misura diretta degli steroidi è metodologicamente inadeguata per ricerche cliniche di alta qualità

(Handelsman DJ, Wartovsky L, JCEM 2013)

Caratteristiche della LC-SM/SM

54 anni per steroidi: Brewer e Sickmann, 1975

Metodo di riferimento per la misura degli steroidi

Multianalyte profiling

Metodo costoso dal punto di vista strumentale

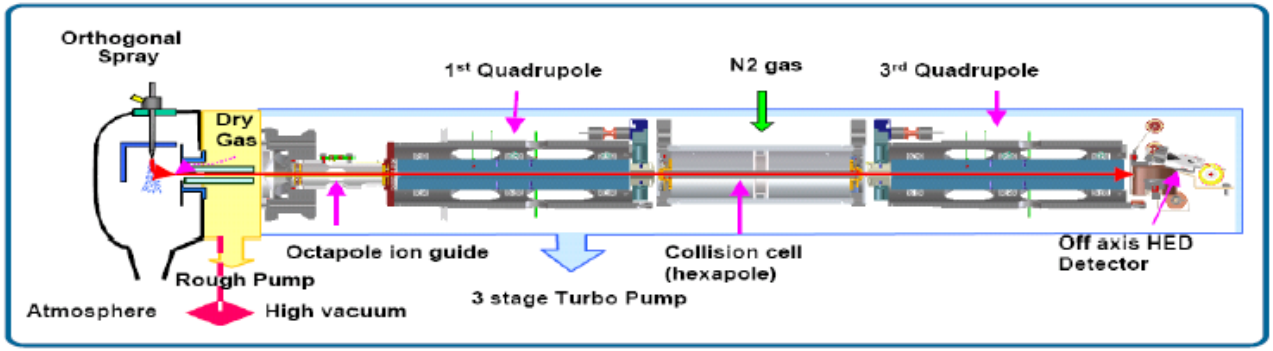
Necessità di expertise del personale tecnico

Bassa (?) automazione

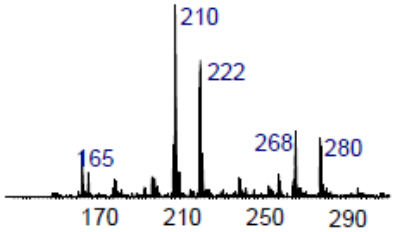
La pratica corrente di laboratorio

- Consolidamento e ‘Big Laboratories’ (provinciali)
- Elevati volumi di prestazioni
- Necessità dell’alta automazione
- Il laboratorio specialistico (nicchie diagnostiche)
  - Test mirati singoli
  - Area di impiego delle nuove tecnologie (tra cui LC-MS/MS)

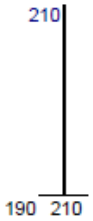
LC-MS/MS



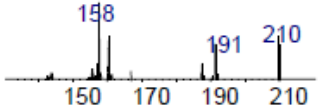
Spectrum with background ions (from ESI)



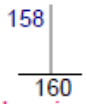
Q1 lets **only** target ion 210 pass through



Collision cell breaks ion 210 apart



Q3 monitors **only** characteristic fragment 158 from ion 210 for quantitation



no chemical background

For Research Use Only.
Not for use in diagnostic procedures.

Integrazione delle tecnologie (IMA e LC-MS/MS)

Test specifici (cortisolo urinario e salivare) per migliorare la specificità (interferenze)

Test di conferma (17-OH Progesterone) per migliorare la specificità

Test per pazienti specifici (testosterone totale)

- Reflex test al di sotto di sensibilità IMA
- Nei prepuberi e nel sesso femminile

Il cortisolo come test diagnostico nell'ipercortisolismo (sindrome di Cushing)

Nieman LK, et al. J. Clin Endocrinol Metab 2008; 93: 1526-40

The Diagnosis of Cushing's Syndrome: An Endocrine Society Clinical Practice Guideline

Lynnette K. Nieman, Beverly M. K. Biller, James W. Findling, John Newell-Price, Martin O. Savage, Paul M. Stewart, and Victor M. Montori

Program on Reproductive and Adult Endocrinology (L.K.N.), National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892; Neuroendocrine Unit/Massachusetts General Hospital (B.M.K.B.), Boston, Massachusetts 02114; Medical College of Wisconsin (J.W.F.), Milwaukee, Wisconsin 53226; University of Sheffield (J.N.-P.), Sheffield S102JF, United Kingdom; William Harvey Research Institute, Queen Mary, University of London (M.O.S.), London EC1M6BQ, United Kingdom; University of Birmingham (P.M.S.), Birmingham B15 2TT, United Kingdom; and Mayo Clinic (V.M.M.), Rochester, Minnesota 55905

Co-sponsoring Association: European Society of Endocrinology

We recommend

- **Urinary free cortisol (UFC; at least two measurements)**
- **Late-night salivary cortisol (LNSC; at least two measurements)**
- Serum cortisol: 1 mg overnight dexamethasone suppression test (oDST)
- Serum cortisol: longer low-dose DST (2 mg/D for 48 hours)

We recommend against:

- Random serum cortisol or plasma ACTH levels
- Urinary 17-ketosteroids
- Insulin tolerance test
- Loperamide test
- DST (8 mg)

Cortisolo libero urinario: metodi di dosaggio

Metodi cromatografici: HPLC, GC/LC-MS/MS

Metodi immunometrici competitivi con estrazione, manuali (RIA, EIA), automatizzati (CLIA, ECLIA, ecc)

Metodi immunometrici competitivi senza estrazione, manuali (RIA, EIA), automatizzati (CLIA, ECLIA, FIA, ecc)

UFC: Analytical pitfalls

Gatti R, et al. Clin Biochem 2009;42:1205-17
Deutschbein T, et al. Horm Metab Res 2013;45:118-23

High variability between methods: CV 32-55%

- Interfering substances require sample pre-treatment
- Common practice of liquid/liquid extraction is **time consuming** and introduces **additional variability**
- Reference values or upper reference limits are highly **method-dependent: LC-MS/MS are approximately 40% of the immunoassay results**

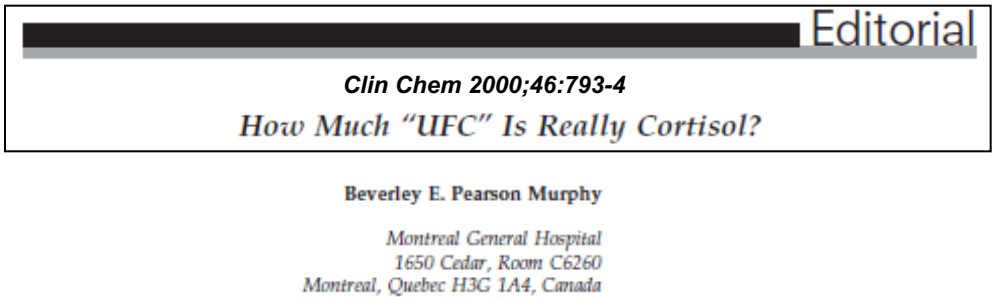
Variability between males and females

- Use of the upper limit of normal (URL) as the criterion for positive test
- Males were repeatedly found to have up to 1.5-fold higher UFC levels than females

INTERFERENZE ANALITICHE DEL DOSAGGIO IMMUNOMETRICO DEL CORTISOLO URINARIO 'CORTISOLO LIBERO O CORTICOIDI LIBERI?'

Analita interferente	% cross-reattività
Cortisone	1-54
Desossicortisolo	8.9-21.6
Corticosterone	0.7-8.0
Prednisolone	2.5-65
Prednisone	0.3-42.9
5-Diidrocortisolo	2.5-30
5-Tetraidrocortisolo	0.2-30

Cumulative data from:
Ching SJ, 2006
Horie H, 2007
Wood L, 2008
Dorizzi R, 2010
Bianchi L, 2019



Editorial

Clin Chem 2000;46:793-4

How Much "UFC" Is Really Cortisol?

Beverley E. Pearson Murphy

Montreal General Hospital
1650 Cedar, Room C6260
Montreal, Quebec H3G 1A4, Canada

UFC: LC-MS/MS

The improved LC-MS/MS method is both sensitive and specific with enhanced analytical performance and clinical diagnostic utility to screen for Cushing syndrome.

The clinical diagnostic sensitivity and specificity were superior to IMA.

El-Farhan N, et al. Ann Clin Biochem 2017;54:308-22

Brossaud J, et al. Clin Chem Lab Med 2018;56:1109-16

Bianchi L, et al. J Endocrinol Invest 2019;42:1299-305

Luo A, et al. Steroids 2019;42:146:65-9

Cortisolo salivare

Data la frequente richiesta contemporanea di UFC e di SC, è opportuno introdurre LC-MS/MS anche per il cortisolo salivare, nonostante IMA forniscano risultati sovrapponibili per l'ipercortisolismo.

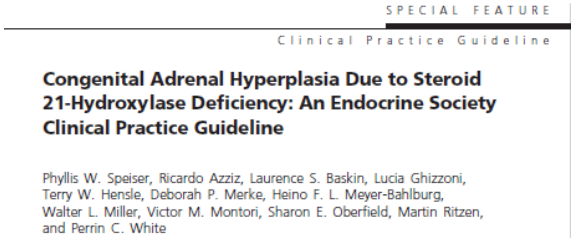
LC-MS/MS presenta maggiore sensibilità analitica rispetto a IMA: circa 50-70% di casi di soggetti sani presentano valori di CS al di sotto del limite di rilevazione di IMA.

LC-MS/MS è preferibile nel caso di ipocortisolismo nel monitoraggio della terapia sostitutiva con glicocorticoidi.

Meszaros k, et al. J Endocrinol Invest 2018;41:315-23

El-Farhan N, et al. 2Ann Clin Biochem 2017;54:308-22

17-OHP
Congenital adrenal hyperplasia
Speiser PW, et al. JCEM 2010;95:4133-60



SPECIAL FEATURE

Clinical Practice Guideline

Congenital Adrenal Hyperplasia Due to Steroid 21-Hydroxylase Deficiency: An Endocrine Society Clinical Practice Guideline

Phyllis W. Speiser, Ricardo Azziz, Laurence S. Baskin, Lucia Ghizzoni, Terry W. Hensle, Deborah P. Merke, Heino F. L. Meyer-Bahlburg, Walter L. Miller, Victor M. Montori, Sharon E. Oberfield, Martin Ritzen, and Perrin C. White

(Newborn screening). We recommend that screening for 21-hydroxylase deficiency … uses a two-tier protocol (initial immunoassay with further evaluation of positive test by liquid chromatography/tandem mass spectrometry).

(Diagnosis after infancy). We recommend obtaining an early morning basement serum 17-OHP in symptomatic individuals.

We recommemd obtaining a complete adrenocortical profile after a cosyntropin stimulation test to make diagnosis in borderline cases.

17-OHP assay methods

- Manual (indirect, direct) immunoassays
  - Radio-immunoassay - RIA (since 1976)
  - Enzyme-immunoassay - EIA (since 1978)
- Automated direct immunoassays
  - Fluoro-immunoassay – FIA (since 1995)
  - Chemiluminescence immunoassay - CLIA (since 2014)
- Liquid chromatography-tandem mass spectrometry (LC-MS) (since 2001)

17-OHP LC-MS/MS: characteristics

Wudy SA, et al. The art of measuring steroids. J Steroid Biochem Mol Biol 2018;179:88-103

Simultaneous measurement of several analytes (steroid profile)

Effective and promising for second-tier testing

High analytical specificity

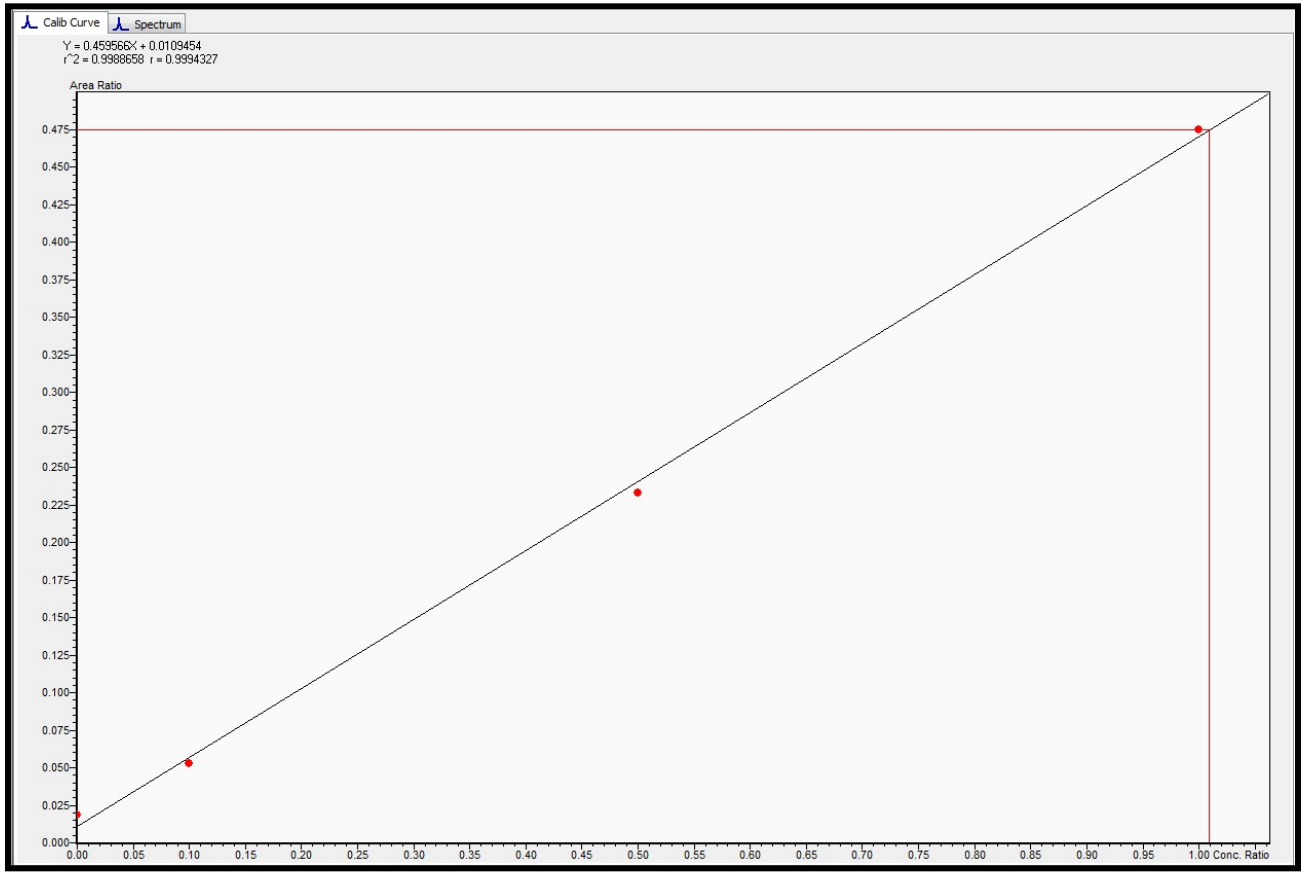
More expensive

Time consuming

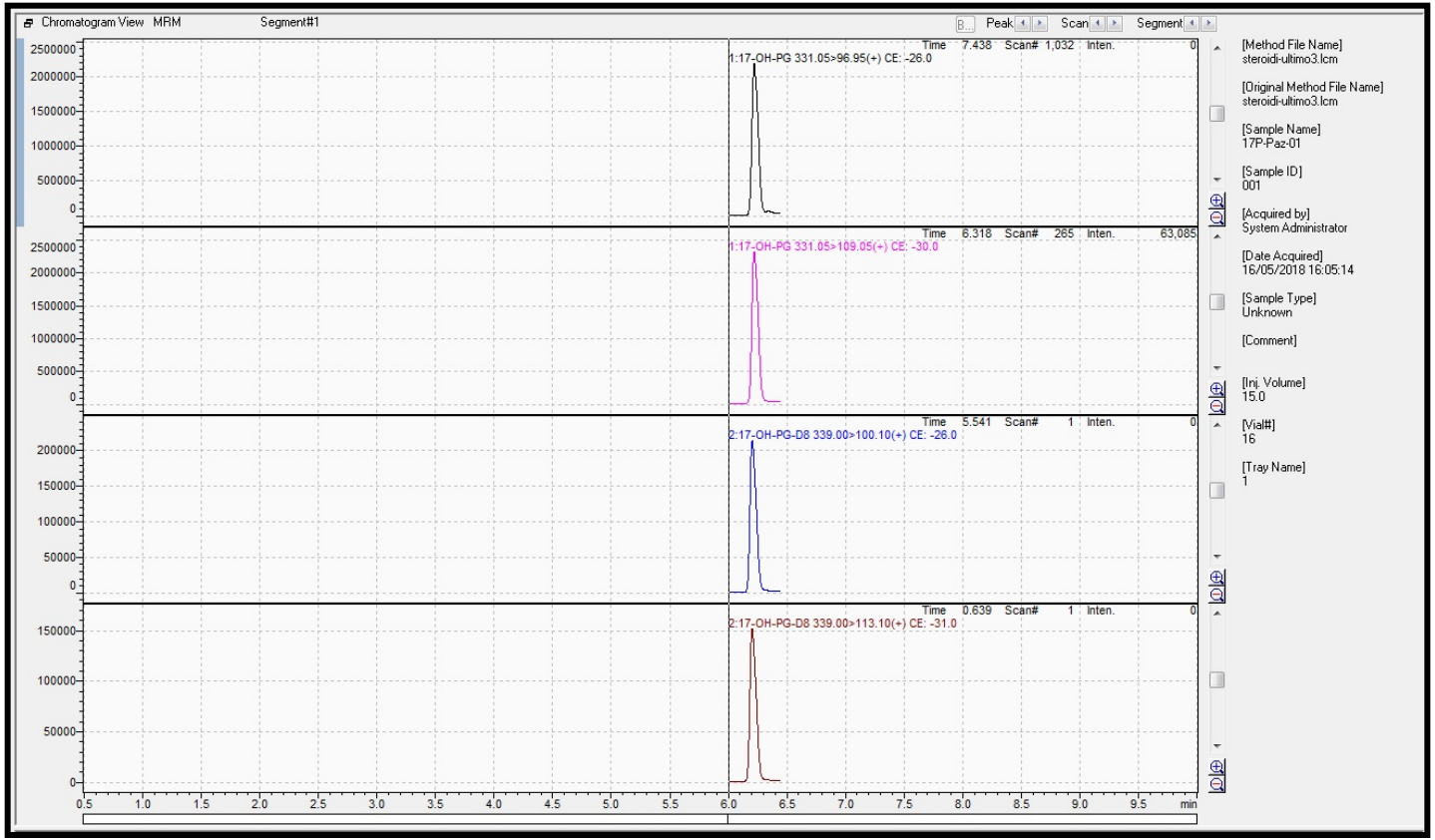
High technical expertise

17-OHP Calibration curve 0-10 ng/mL r = 0.9999568

17-OHP Calibration curve 0-0.5 ng/mL r = 0.9994327



17-OHP


Chromatogram View MRM Segment#1
Peak Scan Segment
Time 7.438 Scan# 1,032 Inten. 0
1:17-OH-PG 331.05>96.95(+) CE: -26.0
Time 6.318 Scan# 265 Inten. 63,085
1:17-OH-PG 331.05>109.05(+) CE: -30.0
Time 5.541 Scan# 1 Inten. 0
2:17-OH-PG-D8 339.00>100.10(+) CE: -26.0
Time 0.639 Scan# 1 Inten. 0
2:17-OH-PG-D8 339.00>113.10(+) CE: -31.0
min
[Method File Name]
steroidi-ultimo3.lcm
[Original Method File Name]
steroidi-ultimo3.lcm
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17P-Paz-01
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System Administrator
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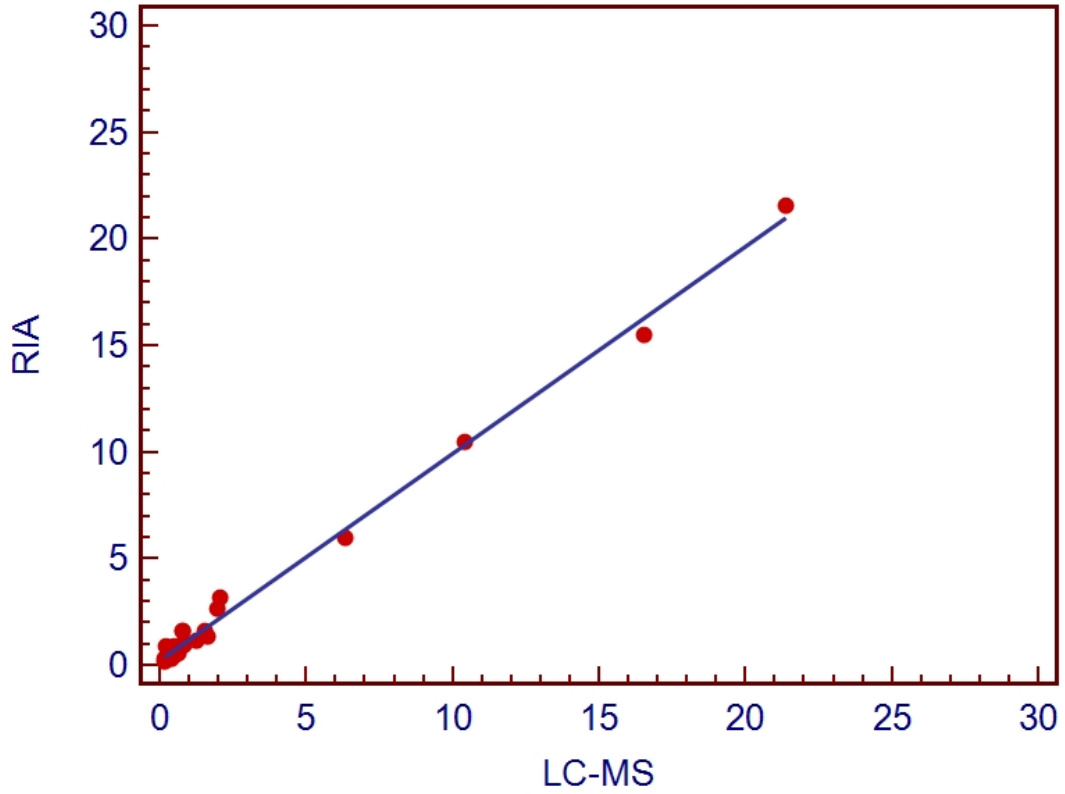
1

17-OHP: LC-MS/MS vs RIA



r: 0.997
CL: 0.9934-0.9987
P <0.0001

Total testosterone in females and children

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

J Clin Endocrinol Metab 2014;99:777-9

LC-MS/MS is a better measurement technique than immunoassay for steroid analysis because it does not suffer from cross-reactivity, the limit of quantitation is lower, and it can multiplex more than one steroid.

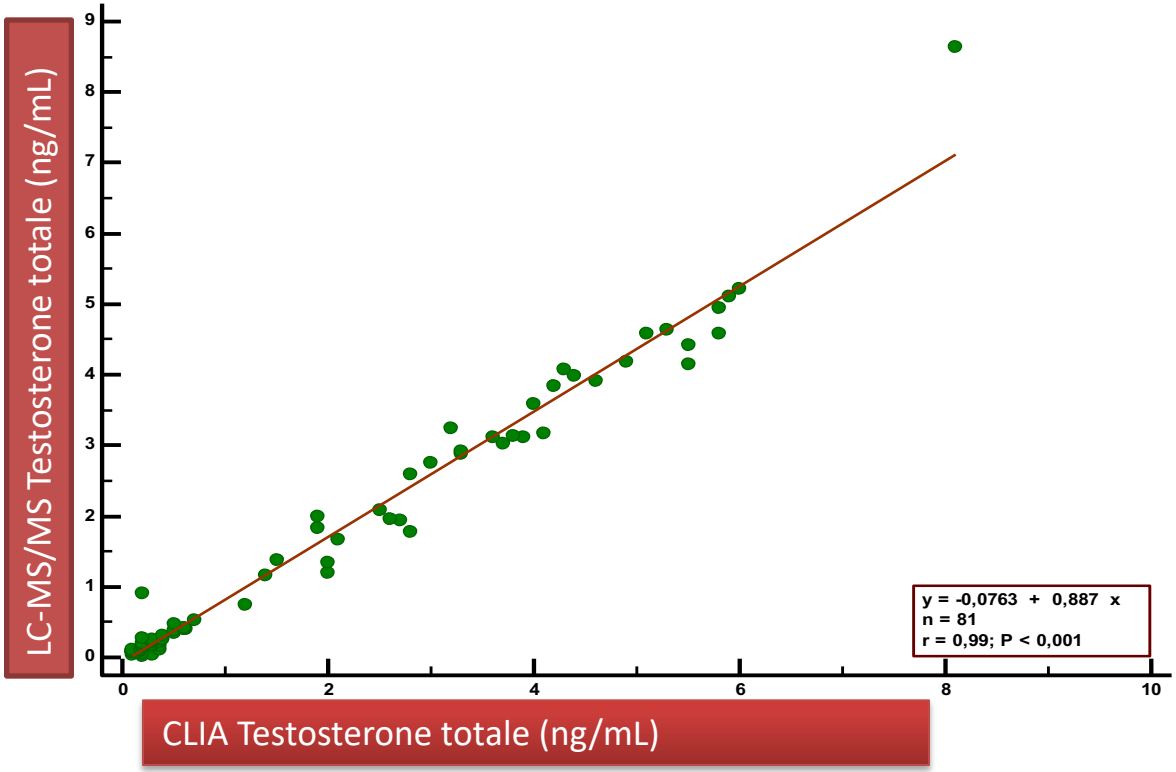
Reflex testing from IMA to LC/MSMS has been suggested when T falls below 100 ng/dL.

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.

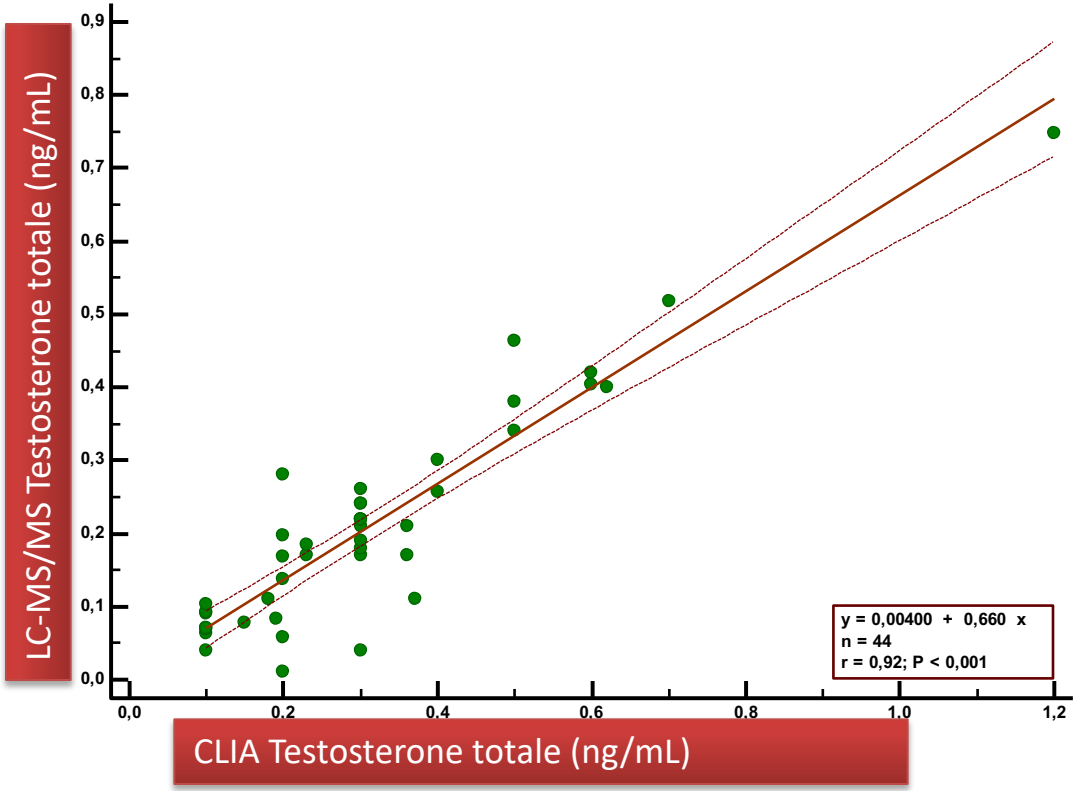
Testosterone IMA-LC-MS/MS

- Campione di 81 pazienti (33 F/48 M)
- Correlazione elevata: 0.99
- Valori LC-MS/MS mediamente inferiori del 15.4-29% nel sesso femminile
- Sensibilità analitica LC-MS/MS decisamente superiore
- Valori LC-MS/MS mediamente inferiori del 5-10% nel sesso maschile, spesso sovrapponibili

Confronto tra medie dei risultati:
CLIA: 1.87 ng/L;
LC-MS/MS: 1.58 ng/L
Delta: 15.4%



Confronto tra medie dei risultati:
CLIA: 0.31 ng/L;
LC-MS/MS: 0.22 ng/L
Delta: 29.0%



Take home messages

- ✓ È giunto il momento di uscire dalla palude e di introdurre in laboratorio la misura corrente degli steroidi con LC-MS/MS
- ✓ I test iniziali possono essere: CLU, CS, 17-OHP, TT
- ✓ LC-MS/MS misura stabilmente gli steroidi a concentrazioni inferiori rispetto a IMA, per i noti problemi di cross-reattività
- ✓ Vanno rigorosamente definiti nuovi intervalli di riferimento, perché stabilmente inferiori