ABC (Altogether to Beat Cushing's syndrome)
"Viaggio alla (ri)scoperta della sindrome di Cushing"
Sorrento, 27 – 30 maggio 2014

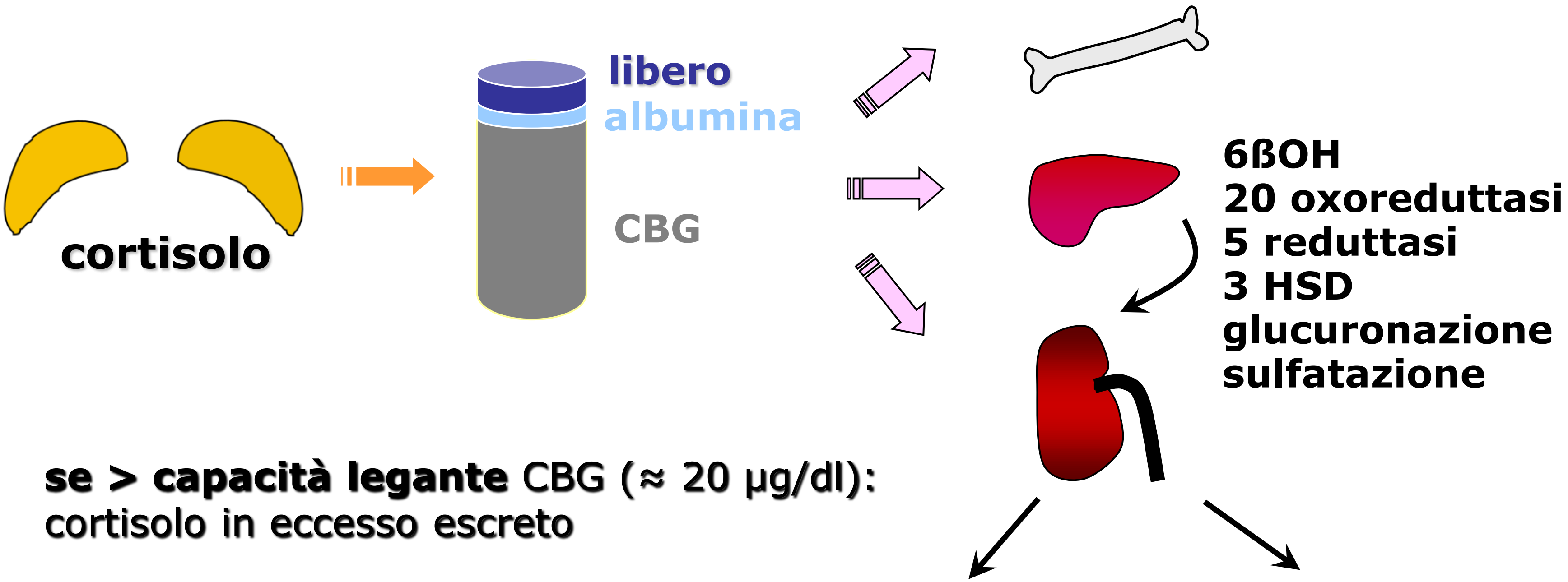
LO SCREENING DELLA SINDROME DI CUSHING: QUALE CORTISOLO?

IL CORTISOLO URINARIO: PASSATO E FUTURO

Emanuela Arvat

Divisione di Endocrinologia Oncologica
Dipartimento di Scienze Mediche
Universita' di Torino

CORTISOLO LIBERO URINARIO

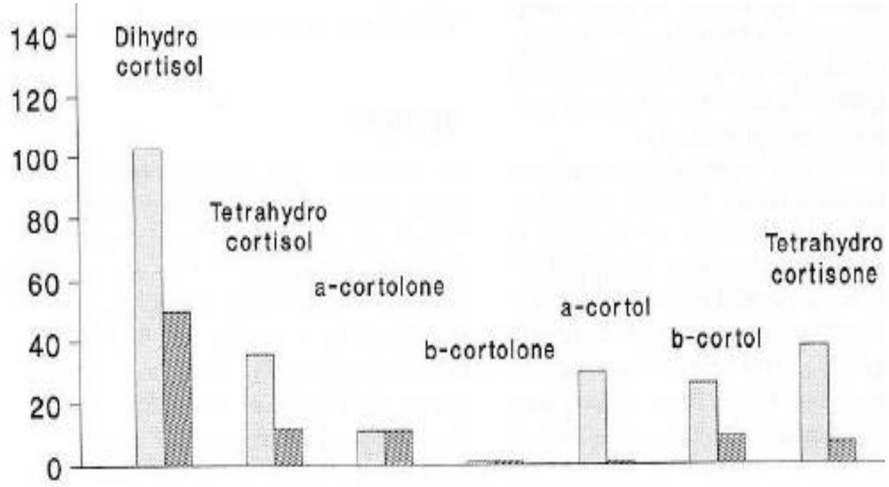


6ßOH
20 oxoreduttasi
5 reduttasi
3 HSD
glucuronazione
sulfatazione

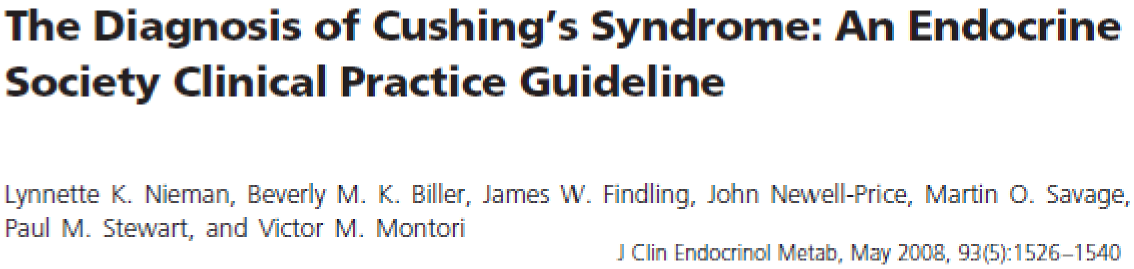
se > capacità legante CBG (≈ 20 µg/dl):
cortisolo in eccesso escreto

UFC

metaboliti



5 tetraidrocortisolo
20 diidrocortisolo
6ß idrocortisolo
glucoronati
etiocolanolone
cortoli/acido cortolico



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

J Clin Endocrinol Metab, May 2008, 93(5):1526–1540

Initial testing

For the initial testing for CS, we recommended one of the following tests based on its suitability for a given patient:

- **UFC (at least two measurements) (different cutoff levels)**
- **1-mg overnight DST (cutoff 1.8 better than 5 μg/dl)**
- **Late-night salivary cortisol (two measurements) (different cutoff levels)**
- **Longer low-dose DST (2 mg/d for 48 h) (cutoff 1.8 μg/dl)**

  Preferable in suspected psychiatric conditions, obesity, alcoholism, diabetes mellitus

…normal test results but CS likely…further evaluation

CS unlikely…reevaluation at 6 months

…abnormal test results…further evaluation

Diagnosis and Management of Cushing's Syndrome: Results of an Italian Multicentre Study*

CECILIA INVITTI, FRANCESCA PECORI GIRALDI, MARTINA DE MARTIN,
FRANCESCO CAVAGNINI, AND THE STUDY GROUP OF THE ITALIAN SOCIETY
OF ENDOCRINOLOGY ON THE PATHOPHYSIOLOGY OF THE
HYPOTHALAMIC-PITUITARY-ADRENAL AXIS†

(*J Clin Endocrinol Metab* 84: 440–448, 1999

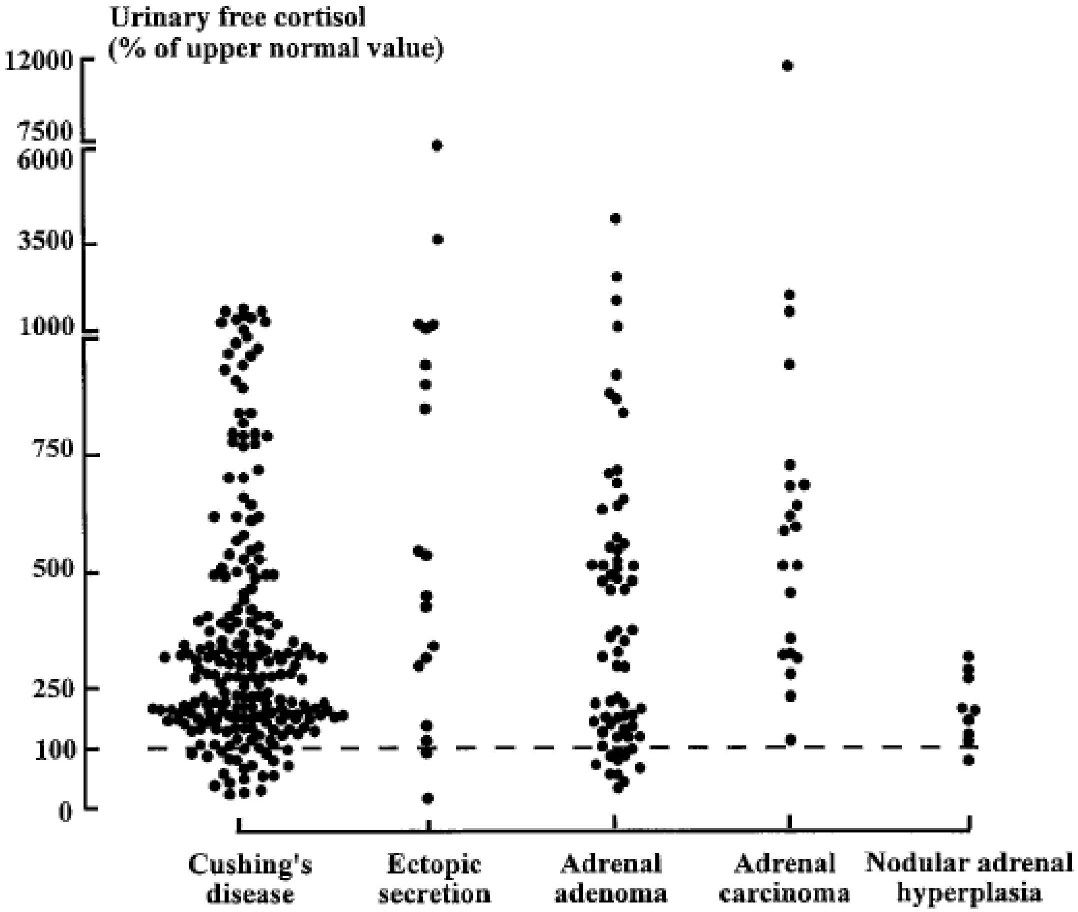
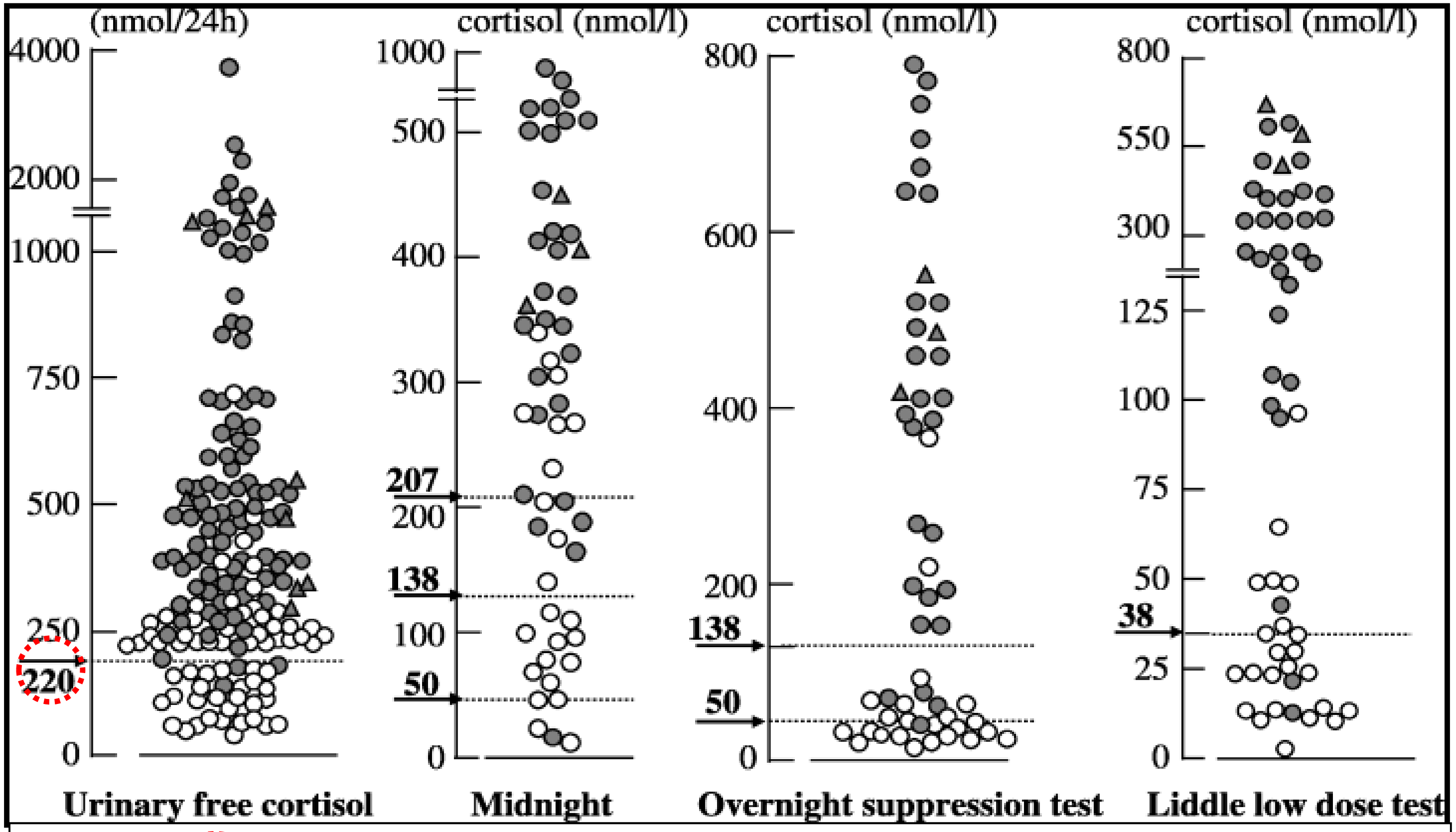


FIG. 1. Distribution of UFC levels, expressed as percent of the upper limit of the normal range, in patients with CS. *Symbols* represent the lowest recorded value among multiple collections.

The dexamethasone-suppressed corticotropin-releasing hormone stimulation test and the desmopressin test to distinguish Cushing's syndrome from pseudo-Cushing's states

Francesca Pecori Giraldi*, Rosario Pivonello,† Alberto Giacinto Ambrogio,* Maria Cristina De Martino,† Martina De Martin,* Massimo Scacchi,* Annamaria Colao,† Paola Maria Toja,* Gaetano Lombardi† and Francesco Cavagnini*

Clinical Endocrinology (2007) 66, 251–257

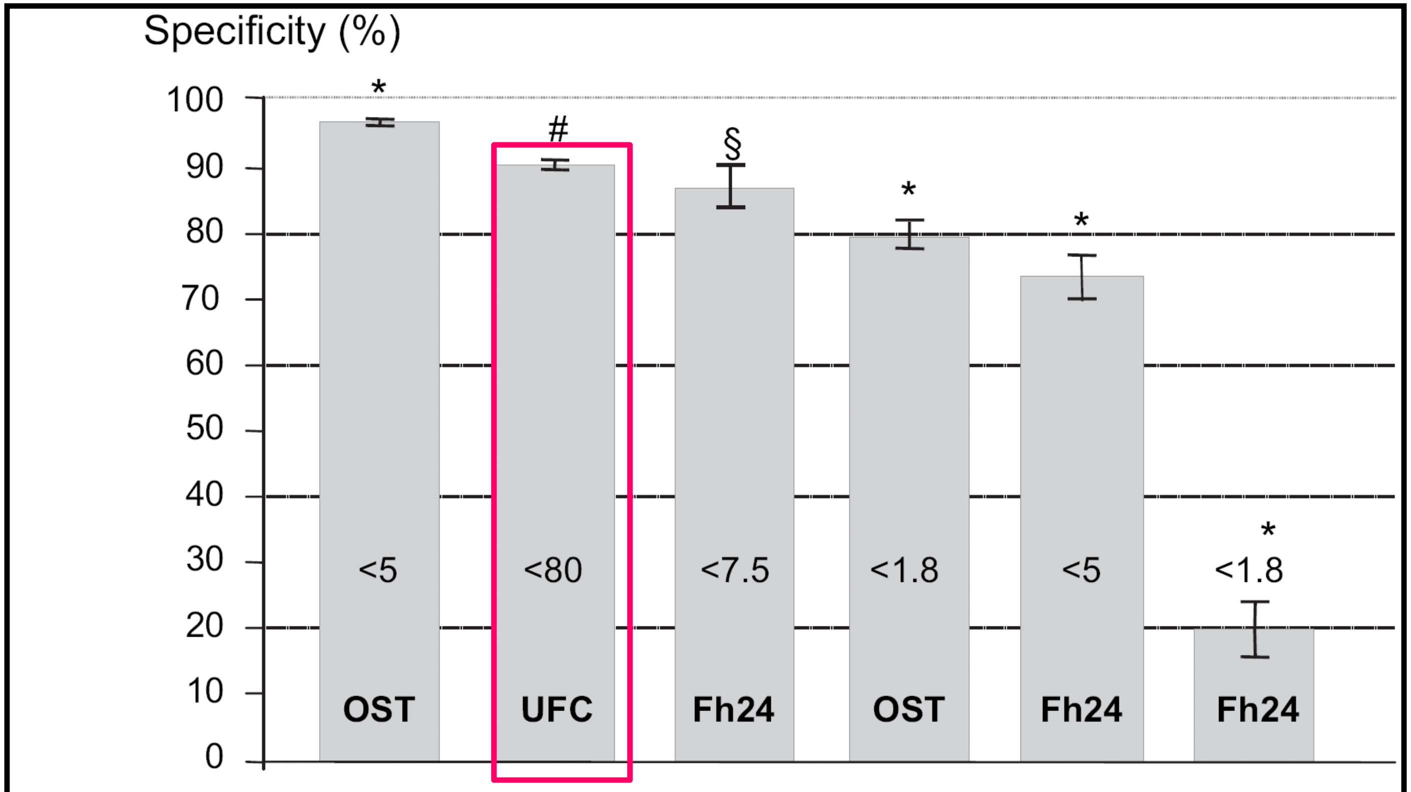


	(≥80 µg/24h)	(1.8 µg/dl)	(1.8 µg/dl)	(1.4 µg/dl)
Sensitivity:	97%	98%	98%	94%
Specificity:	44%	18%	58%	74%

Specificity of First-Line Tests for the Diagnosis of Cushing's Syndrome: Assessment in a Large Series

Francesca Pecori Giraldi, Alberto G. Ambrogio, Martina De Martin, Letizia M. Fatti, Massimo Scacchi, and Francesco Cavagnini

J Clin Endocrinol Metab, November 2007, 92(11):4123–4129



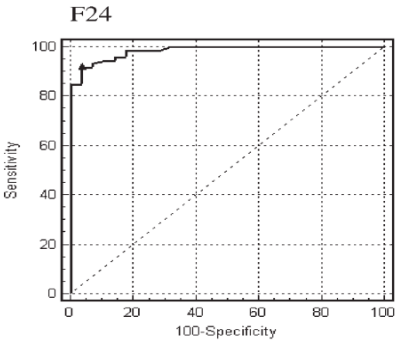
Reported sensitivities

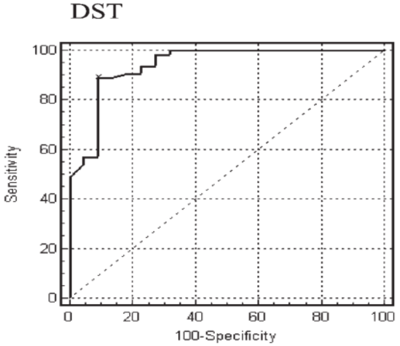
	OST	UFC	Fh24	OST	Fh24	Fh24
minimum	82% [16]	91% [2]	90% [2]	98% [40]	95% [2]	99% [14]
maximum	100% [23]	96% [39]	96% [14]	100% [9]	100% [3]	100% [15]

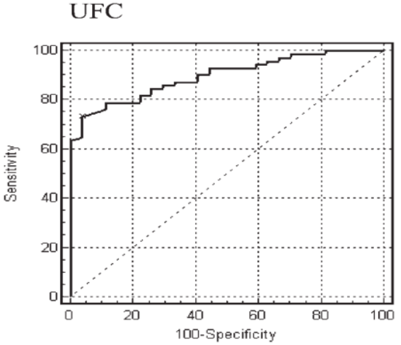
Evaluation of the effectiveness of midnight serum cortisol in the diagnostic procedures for Cushing's syndrome

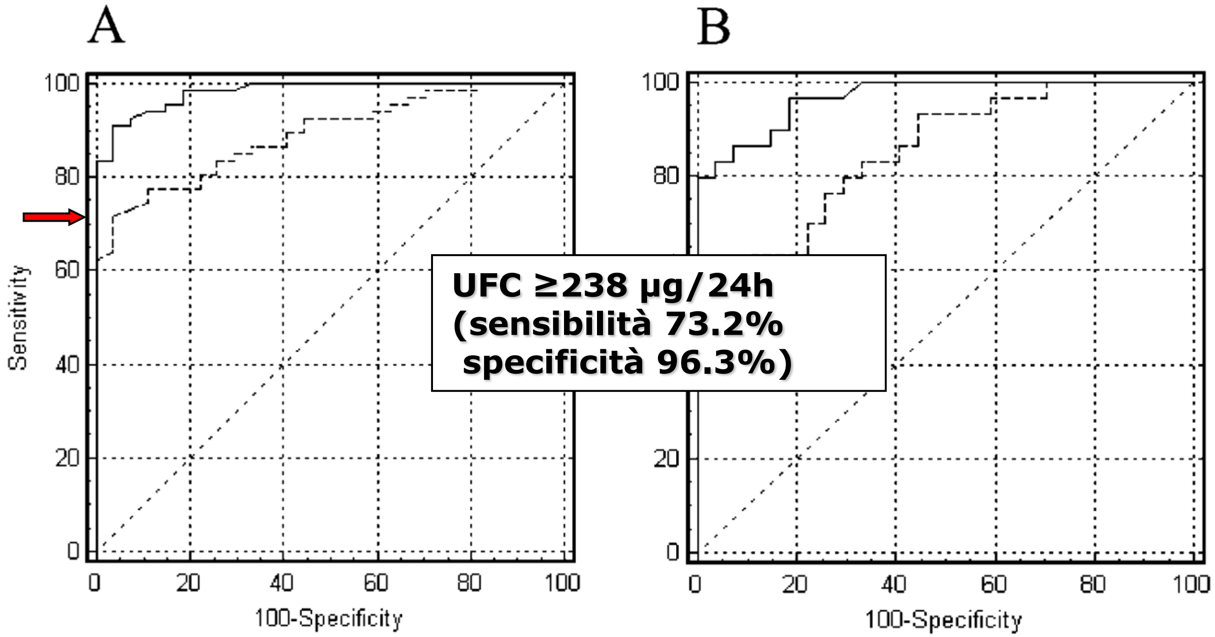
Giuseppe Reimondo, Barbara Allasino, Silvia Bovio, Piero Paccotti, Alberto Angeli and Massimo Terzolo

European Journal of Endocrinology (2005) **153** 803–809









UFC ≥238 µg/24h
(sensibilità 73.2%
specificità 96.3%)

(A) Comparison between the ROC curve of F24 (solid line) and UFC (dashed line) in the whole series. (B) Comparison between the ROC curve of F24 (solid line) and UFC (dashed line) in the subgroup of patients with mild Cushing's syndrome.

SENSIBILITA' & SPECIFICITA'

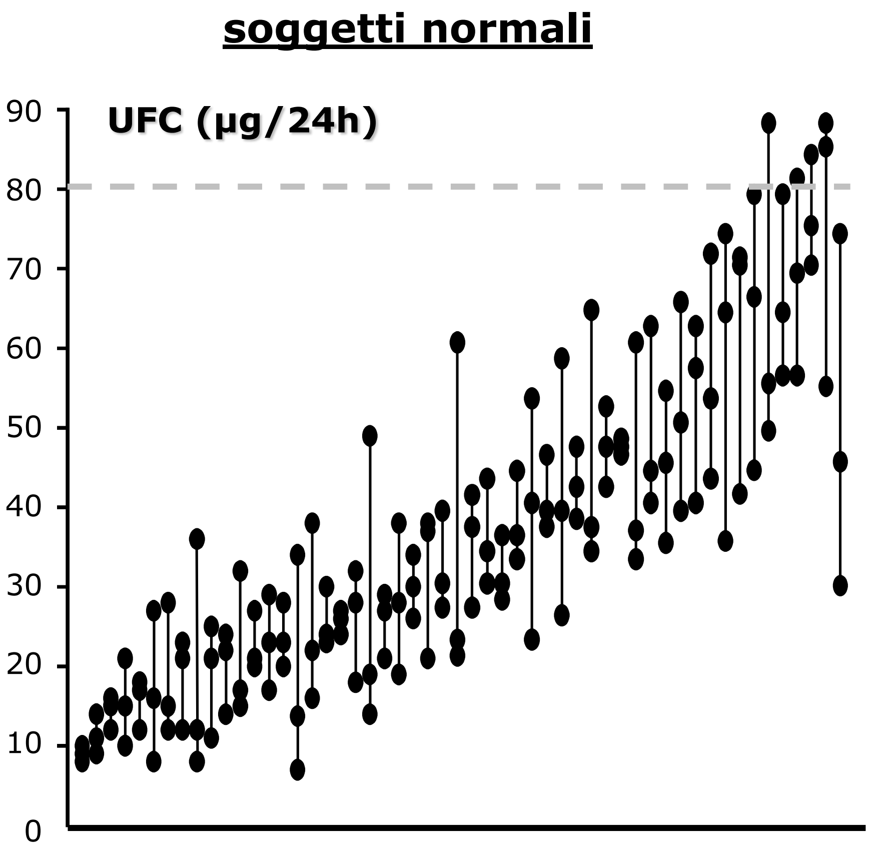
lavoro	Cut-off	metodo	Cushing	normali	obesi	sosp Cushing	Sens	Spec
Kley 1978	100 µg/24h	CPB	69	317			97%	99%
Crapo 1979	181 µg/24h	CR	248	262	104	32	97%	99%
Zadik 1980	97 µg/24h	CPB	13	94	95		94%	97%
Vidal 1983	95 µg/24h	CPB	26	9		19	96%	96%
Contreras 1986	100 µg/24h	CPB	14	9		19	100%	100%
Mengden 1992	120 µg/24h	CPB	48			23	100%	98%
Friedman 1995	76 µg/24h	RIA	20	9		161	100%	32%
Papanicolaou 1998							45%	100%
Görges 1999							97%	92%
Moro 2000							92%	66%
Papanicolaou 2002							56%	100%
Putignano 2003	80 µg/24h	RIA	41	20	16	140	98%	85%
Yaneva 2004	90 µg/24h	RIA	63				100%	100%
Viardot 2005	183 µg/24h	RIA	12				100%	100%
Holleman 2005	65 µg/24h	HPLC	16			45	94%	93%
Reimondo 2005	238 µg/24h	RIA	78				73%	96%
Ching 2006	60 µg/24h	HPLC	22				100%	77%

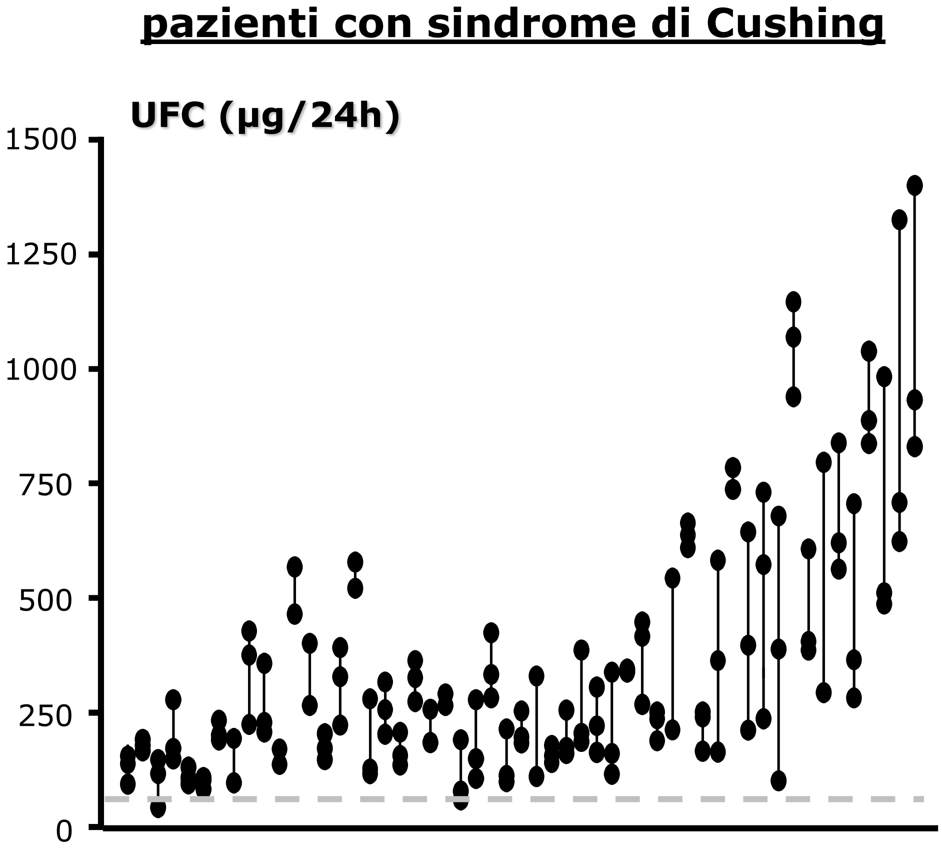
Sensibilità 45-71% a 100% specificità
Specificità 45-73% a 100% sensibilità

Grossman et al, 2011

LR+ 10.6 (5-20); LR- 0.16 (0.08-0.33) *(Elamin 2008)*

VARIABILITA' dell'ESCREZIONE di UFC





Variabilità individuale 41-46% da giorno a giorno (***Deutschbein 2011***)

Limitations of nocturnal salivary cortisol and urine free cortisol in the diagnosis of mild Cushing's syndrome

Srividya Kidambi[1,2], Hershel Raff[1,2] and James W Findling[1,2]

European Journal of Endocrinology (2007) **157** 725–731

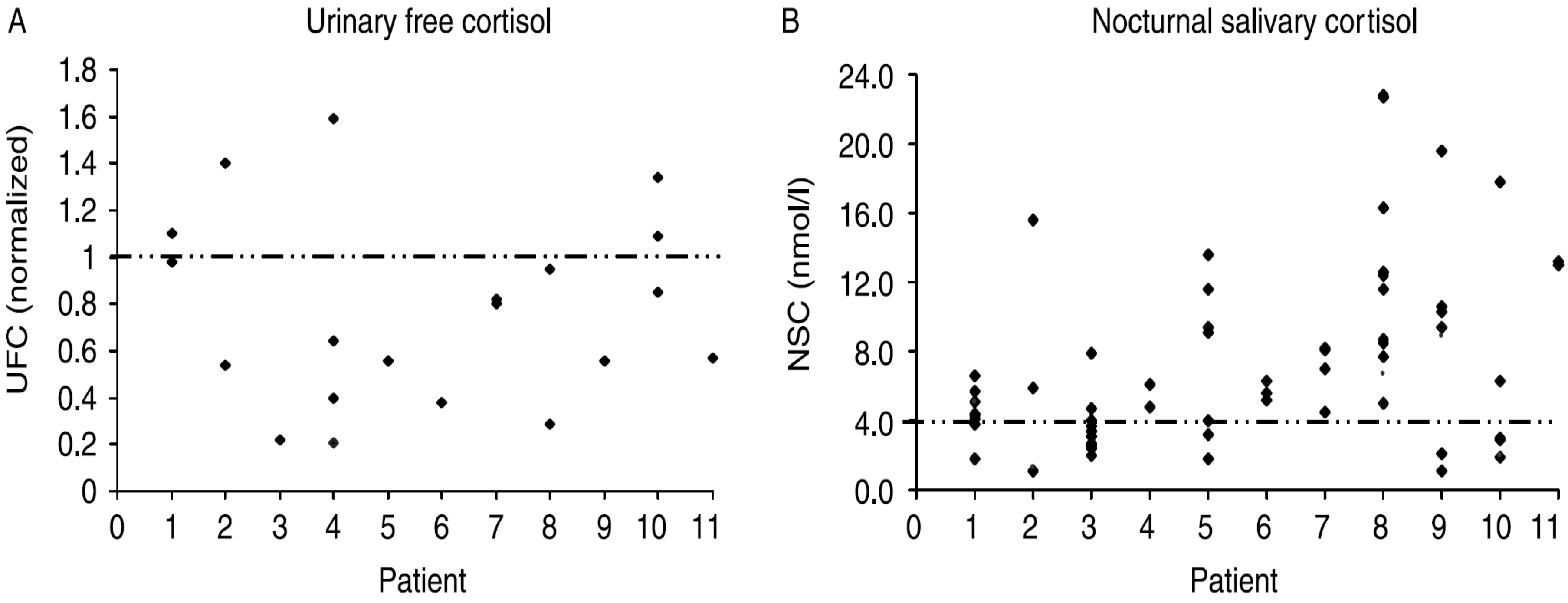


Figure 1 (A) Twenty-four-hour urine free cortisol (UFC) in 11 patients with surgically proven Cushing's syndrome. The data were normalized by the dividing UFC of the subject by the upper limit of the UFC assay reference range (indicated by the dotted horizontal line). (B) Nocturnal salivary cortisol (NSC) levels. The dotted line indicates the upper limit of the reference range (4.2 nmol/l (0.15 mcg/dl)). To convert nmol/l to mcg/dl, divide by 27.59.

Subclinical hypercortisolism: correlation between biochemical diagnostic criteria and clinical aspects

V. Morelli*, B. Masserini*, A. S. Salcuni*·†, C. Eller-Vainicher*, C. Savoca*, R.Viti†, F. Coletti‡, G. Guglielmi§, C. Battista†, L. Iorio¶, P. Beck-Peccoz*, B. Ambrosi¶, M. Arosio‡, A. Scillitani† and I. Chiodini*

Clinical Endocrinology (2010) 73, 161–166

Criteria	Sensitivity	Specificity	Accuracy	*P*
I (1 mg-DST >49·7 nmol/l)	71·4	49·5	51·5	NS
II (1 mg-DST >82·8 nmol/l)	52·4	81·4	78·8	0·001
III (1 mg-DST >137·9 nmol/l)	23·8	93·3	87·0	0·019
IV (elevated UFC)	42·9	80·0	76·6	0·021
V (low ACTH)	52·4	60·5	59·7	NS
VI (1 mg-DST >49·7 nmol/l + low ACTH)	42·9	72·4	69·7	NS
VII (1 mg-DST >82·8 nmol/l + low ACTH)	33·3	86·7	81·8	0·024
VIII (1 mg-DST >137·9 nmol/l + low ACTH)	14·3	94·8	87·4	NS
IX (1 mg-DST >49·7 nmol/l + elevated UFC)	23·8	87·1	81·4	NS
X (1 mg-DST >82·8 nmol/l + elevated UFC)	19·0	93·8	87·0	NS
XI (1 mg-DST >137·9 nmol/l + elevated UFC)	9·5	97·6	89·6	NS
XII (2 of: 1 mg-DST >49·7 nmol/l, elevated UFC, low ACTH)	66·7	62·9	63·2	0·010
XIII (2 of: 1 mg-DST >82·8 nmol/l, elevated UFC, low ACTH)	61·9	77·1	75·8	0·001
XIV (2 of: 1 mg-DST >137·9 nmol/l, elevated UFC, low ACTH)	42·9	86·2	82·3	0·002
XV (1 mg-DST >82·8 nmol/l + elevated UFC or low ACTH)	47·6	83·8	80·5	0·002

Significantly high level of late-night free cortisol to creatinine ratio in urine specimen in patients with subclinical Cushing's syndrome

Tsuguka Shiwa*, Kenji Oki*, Kiminori Yamane†, Masayasu Yoneda*, Tomokazu Awaya*, Shuhei Nakanishi* and Nobuoki Kohno*

Clinical Endocrinology (2013) **79**, 617–622

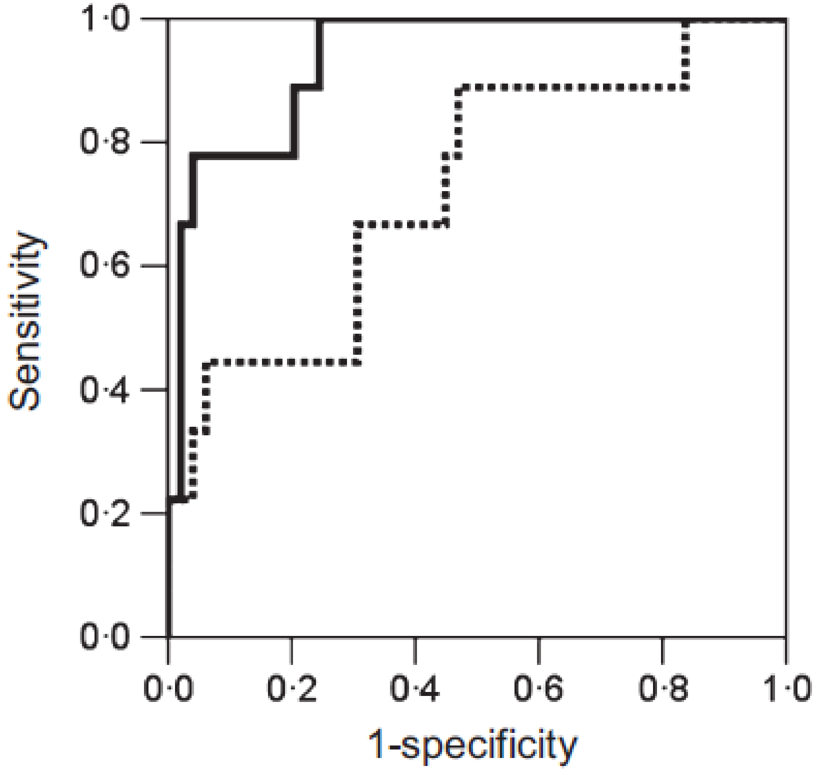


Fig. 3 The areas under the receiver operating characteristic (ROC) curves of endocrinological tests for diagnosis of subclinical Cushing's syndrome. The areas under the ROC curves of late-night urinary free cortisol to creatinine ratio (UFCCR) and 24-h urinary free cortisol (UFC) were 0·937 (0·865–1·008) and 0·726 (0·874–0·999), respectively. Solid line: late-night UFCCR; dotted line: 24-h UFC.

PROBLEMATICHE DEL DOSAGGIO UFC

PRE-ANALITICHE

Quantità di liquidi assunti (>5L → FP)

Raccolta del campione (dosaggio creatininuria)

Filtrato glomerulare (<60 ml/min → FN)

Variabilità intrinseca della secrezione di cortisolo

Condizioni di iperattivazione funzionale dell'asse HPA

PROBLEMATICHE DEL DOSAGGIO UFC

ANALITICHE

METODICHE DI DOSAGGIO

METODICHE IMMUNOCHIMICHE

Immunoradiometriche
Immunoenzimatiche
- dirette
- estrattive

Cross-reattività

Concentrazione di cortisolo nelle urine è molto più bassa di quella di altre molecole steroidee interferenti

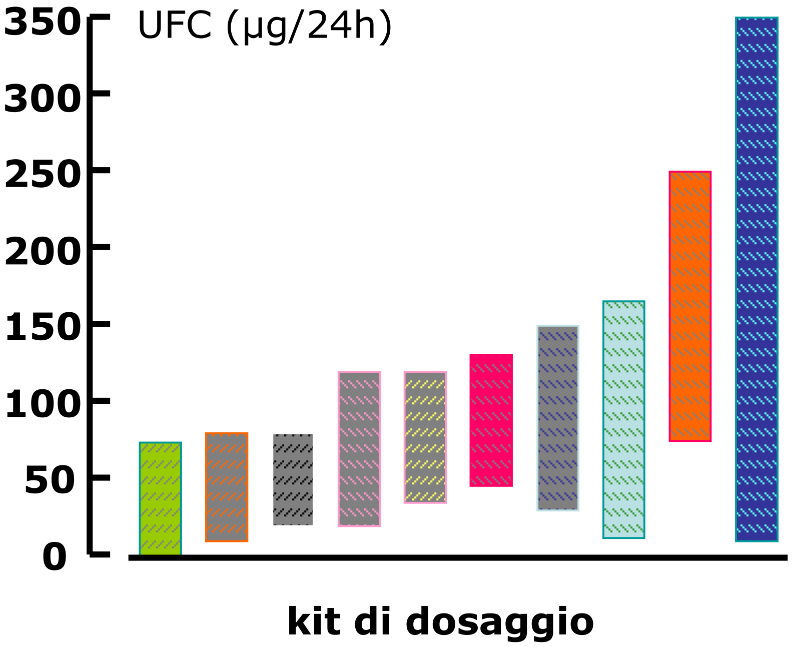
Gli steroidi interferenti sono maggiori rispetto al plasma

Steroid	Cross-reactivity (%)	
Cortisol	100.0	100
Progesterone	14.8	0.02
17-OH-Progesterone	4.5	NR
11-Deoxycorticosterone	4.3	0.26
11-Deoxycortisol	28.3	11.4
Corticosterone	9.8	0.94
18-OH-Deoxycorticosterone	0.7	NR
Cortisone	23.0	0.98
Aldosterone	0.9	0.03
18-OH-Corticosterone	4.3	NR
21-Deoxycortisol	100.0	NR
6-OH-Cortisol	0.3	NR
Prednisone	9.6	2.3
Dexamethasone	0.01	0.04
Tetrahydrocortisol	<0.01	0.34
Tetrahydro-11-deoxycortisol	<0.01	NR
Tetrahydrocorticosterone	<0.01	NR
Testosterone	1.1	NR
Estriol	<0.01	0.01

(Schöneshöfer 1980) *Coat-a-count*

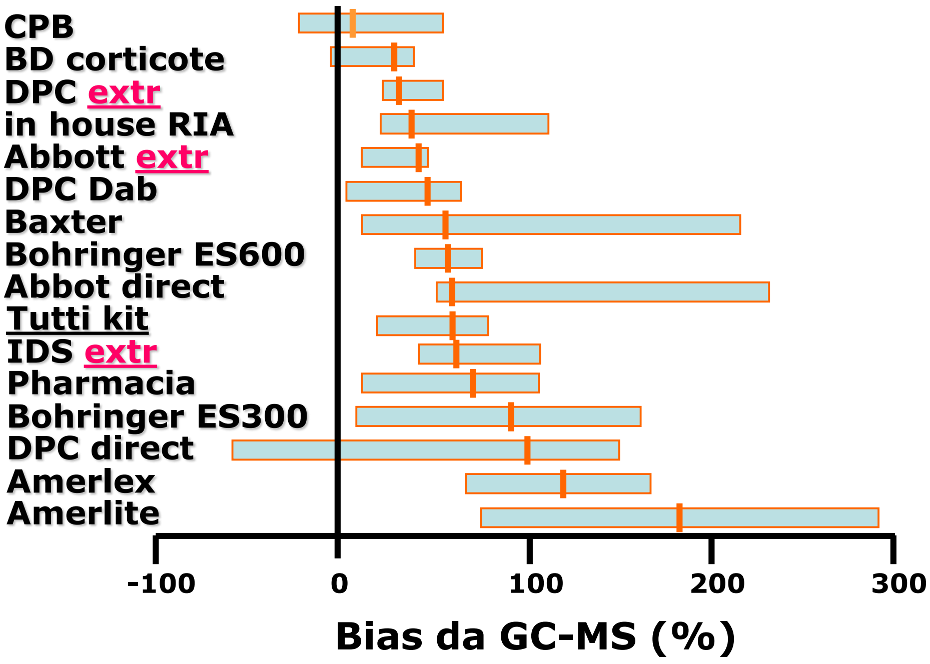
METODICHE IMMUNOCHIMICHE

RANGE DI NORMA



Gruppo di studio HPA

ACCURATEZZA



UKNEQAS - 1998

METODICHE IMMUNOCHIMICHE

SVANTAGGI

Interferenza con altri metaboliti:
→ sovrastima UFC, valori > 2-3 vs cromatografia

Differenti metodiche, range di normalità, cut-off:
→ non comparabilità dei dosaggi

ACCURATEZZA NON OTTIMALE (bassa specificità)

PASSATO ? **ANCORA PRESENTE ……**

VANTAGGI

Semplicità del metodo (automatizzato, utilizzo in routine…)

Elevata produttività analitica

Costo non elevato

FUTURO?

METODICHE CROMATOGRAFICHE

- cromatografia liquida con rilevazione ultravioletti (LC-UV)
- cromatografia liquida ad elevata prestazione e rilevazione ultravioletti (HPL
- cromatografia liqu
- cromatografia liqu (LC-MS/MS)
- cromatografia gas

Assente cross-reattività

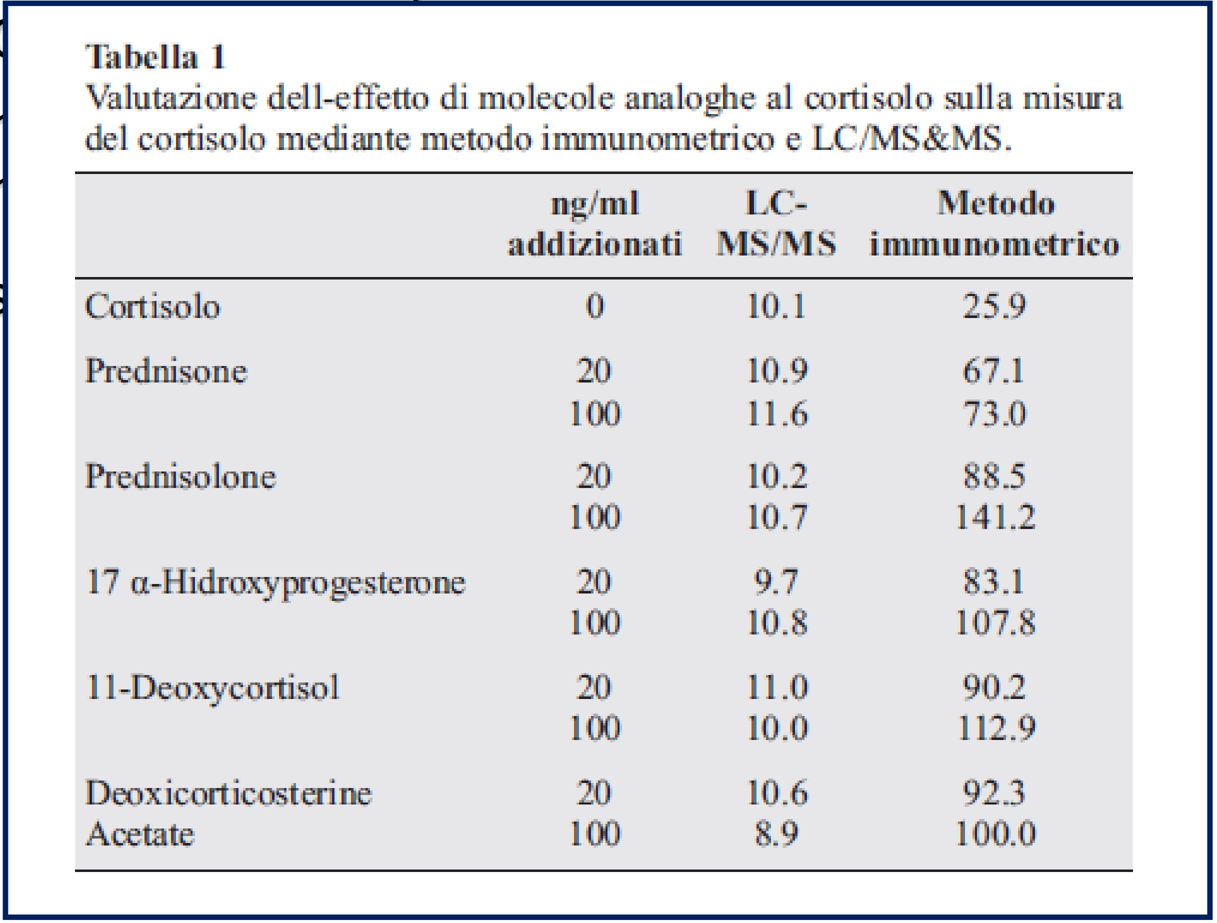


Tabella 1
Valutazione dell-effetto di molecole analoghe al cortisolo sulla misura del cortisolo mediante metodo immunometrico e LC/MS&MS.

	ng/ml addizionati	LC-MS/MS	Metodo immunometrico
Cortisolo	0	10.1	25.9
Prednisone	20	10.9	67.1
	100	11.6	73.0
Prednisolone	20	10.2	88.5
	100	10.7	141.2
17 α-Hidroxyprogesterone	20	9.7	83.1
	100	10.8	107.8
11-Deoxycortisol	20	11.0	90.2
	100	10.0	112.9
Deoxicorticosterine	20	10.6	92.3
Acetate	100	8.9	100.0

Novel liquid chromatography tandem mass spectrometry (LC-MS/MS) methods for measuring steroids

Brian G. Keevil, BSc, MSc, FRCPath, Professor *

Best Practice & Research Clinical Endocrinology & Metabolism 27 (2013) 663–674

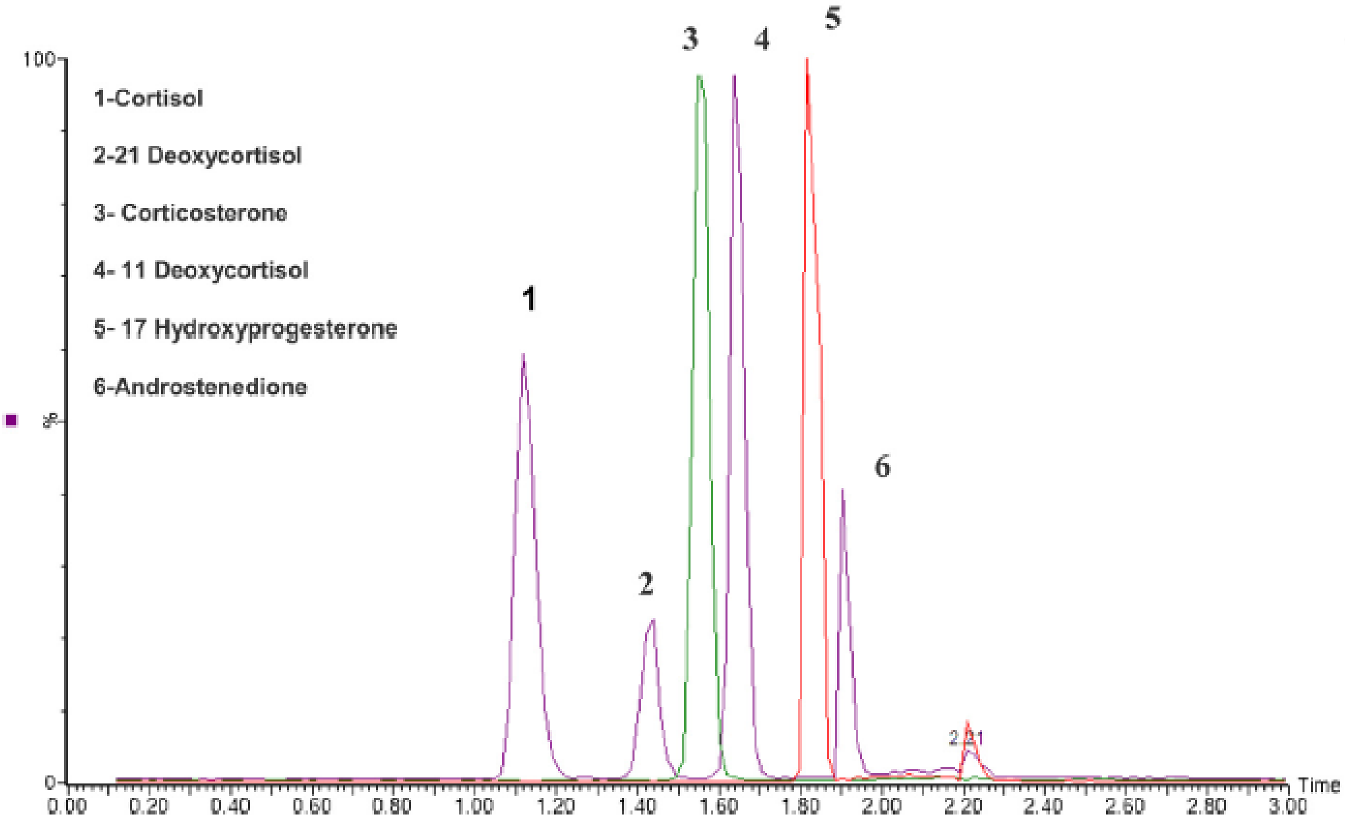


Fig. 1. Separation of some clinically important adrenal steroids using LC-MS/MS. Isobaric compounds 21 deoxycortisol (DOC), Corticosterone and 11 DOC are baseline separated during a 3 min run time.

Urine free cortisol analysis by automated immunoassay and high-performance liquid chromatography for the investigation of Cushing's syndrome

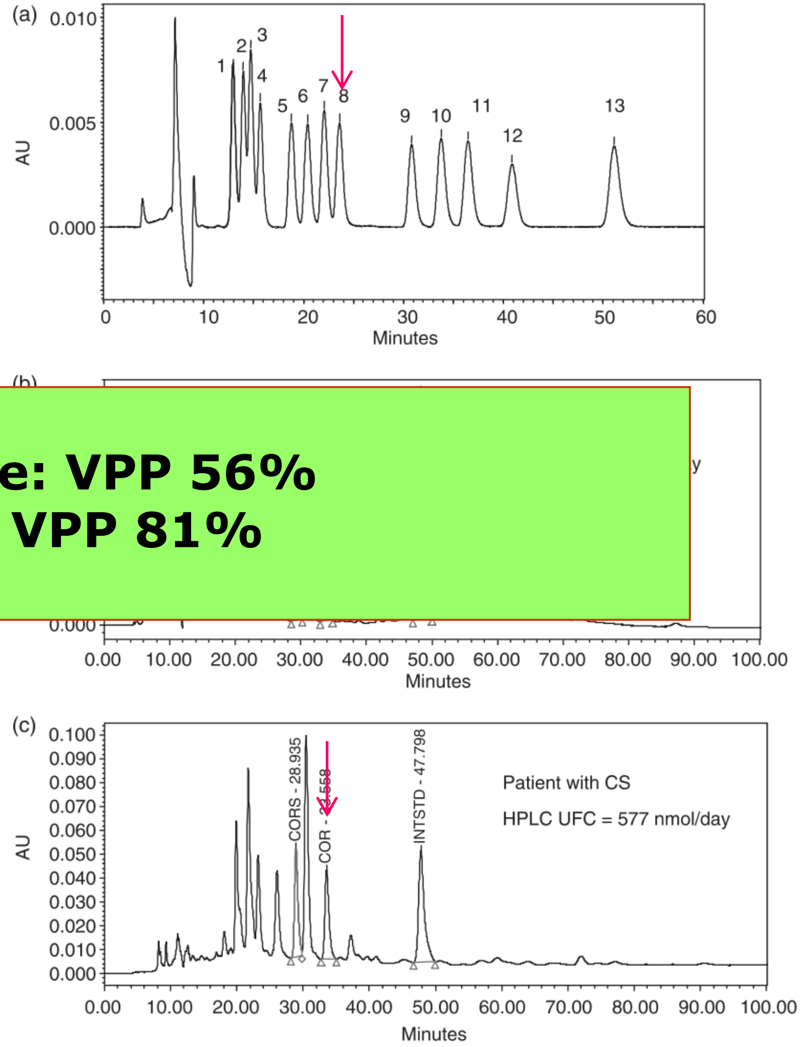
Ann Clin Biochem 2006; **43:** 402–407

Simon Y L Ching[1], Ee Mun Lim[1], John Beilby[1], Chotoo Bhagat[1], Enrico Rossi[1], John P Walsh[2] and Peter Pullan[2]

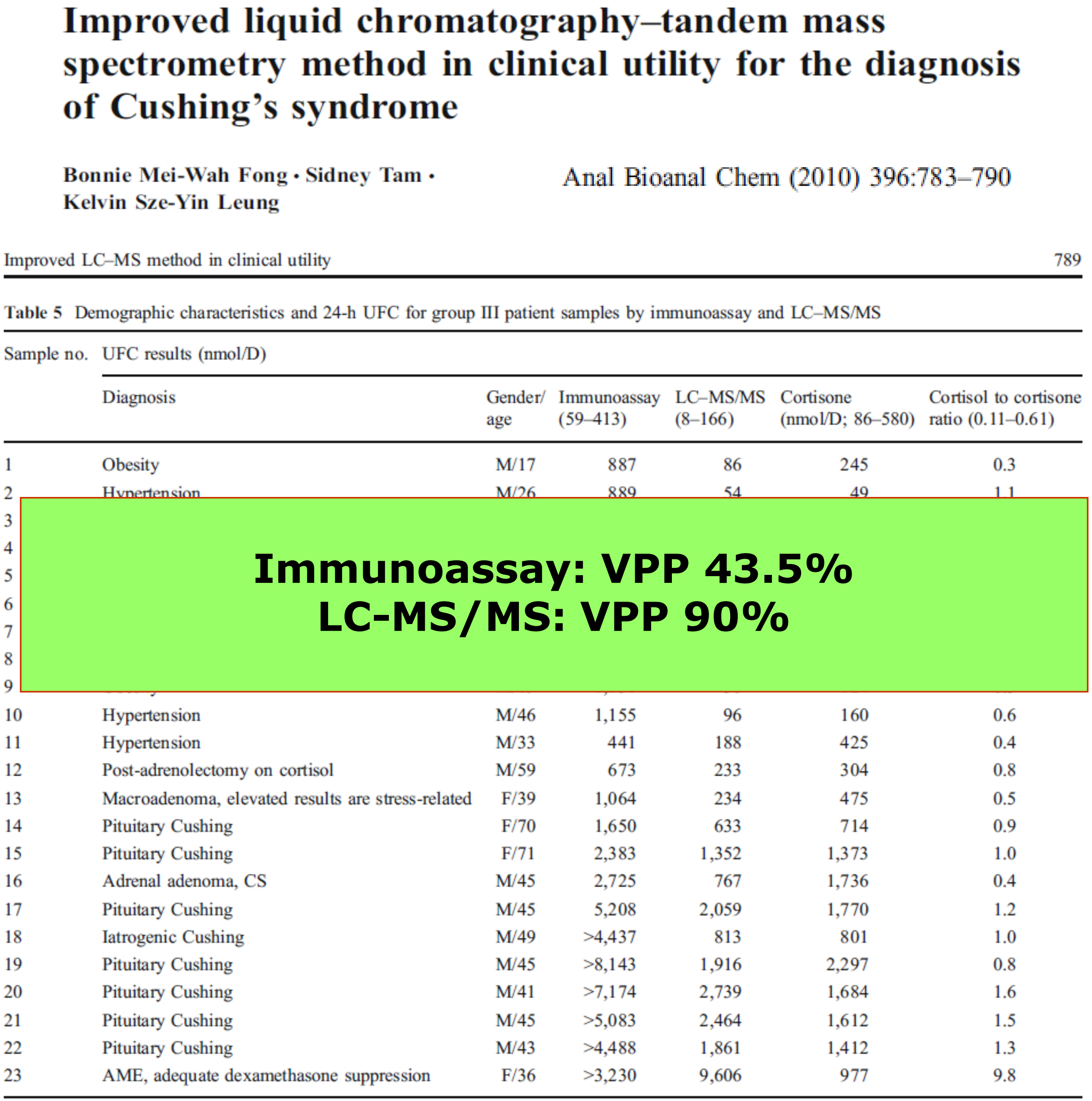
Table 1 *Demographic characteristics and 24-h urine free corticoids by Immulite:2000 and urine free cortisol by HPLC in 22 patients with surgically proven Cushing's syndrome*

			24 h urine results (nmol/day)	
Type of CS	Age years	Sex	Urine free corticoids immulite (<900)	Urine free cortisol HPLC (<170)
Pituitary	43	F	1300	320
Pituitary	54	F	1800	350
Pituitary	26	F	2000	430
Pituitary	40	F	2300	470
Pituitary				
Pituitary				
Pituitary				
Pituitary				
Pituitary				
Pituitary				
Pituitary				
Pituitary	24	F	3000	1280
Pituitary	37	F	4000	1700
Pituitary	47	M	4000	1800
Pituitary	59	F	1100	Interference*
Adrenal Adenoma	42	F	2500	740
Adrenal Adenoma	37	F	2700	940
Adrenal Adenoma	73	M	4700	1170
Adrenal Adenoma	41	F	2300	1220
Adrenal Carcinoma	72	F	3800	760
Ectopic ACTH	75	F	2900	700
Ectopic ACTH	11	F	19,000	10,400

*Interference prevented quantitation of urine free cortisol. Peak absorbance and shape indicated an elevated result



Immulite: VPP 56%
HPLC: VPP 81%



Improved liquid chromatography–tandem mass spectrometry method in clinical utility for the diagnosis of Cushing's syndrome

Bonnie Mei-Wah Fong • Sidney Tam • Kelvin Sze-Yin Leung

Anal Bioanal Chem (2010) 396:783–790

Table 5 Demographic characteristics and 24-h UFC for group III patient samples by immunoassay and LC–MS/MS

Sample no.	UFC results (nmol/D)					
	Diagnosis	Gender/ age	Immunoassay (59–413)	LC–MS/MS (8–166)	Cortisone (nmol/D; 86–580)	Cortisol to cortisone ratio (0.11–0.61)
1	Obesity	M/17	887	86	245	0.3
2	Hypertension	M/26	889	54	49	1.1
3						
4						
5						
6						
7						
8						
9						
10	Hypertension	M/46	1,155	96	160	0.6
11	Hypertension	M/33	441	188	425	0.4
12	Post-adrenolectomy on cortisol	M/59	673	233	304	0.8
13	Macroadenoma, elevated results are stress-related	F/39	1,064	234	475	0.5
14	Pituitary Cushing	F/70	1,650	633	714	0.9
15	Pituitary Cushing	F/71	2,383	1,352	1,373	1.0
16	Adrenal adenoma, CS	M/45	2,725	767	1,736	0.4
17	Pituitary Cushing	M/45	5,208	2,059	1,770	1.2
18	Iatrogenic Cushing	M/49	>4,437	813	801	1.0
19	Pituitary Cushing	M/45	>8,143	1,916	2,297	0.8
20	Pituitary Cushing	M/41	>7,174	2,739	1,684	1.6
21	Pituitary Cushing	M/45	>5,083	2,464	1,612	1.5
22	Pituitary Cushing	M/43	>4,488	1,861	1,412	1.3
23	AME, adequate dexamethasone suppression	F/36	>3,230	9,606	977	9.8

Immunoassay: VPP 43.5%
LC-MS/MS: VPP 90%

Detection of synthetic glucocorticoids by liquid chromatography-tandem mass spectrometry in patients being investigated for Cushing's syndrome

Ann Clin Biochem 2011; **48**: 542–549.

N K Djedovic and S J Rainbow

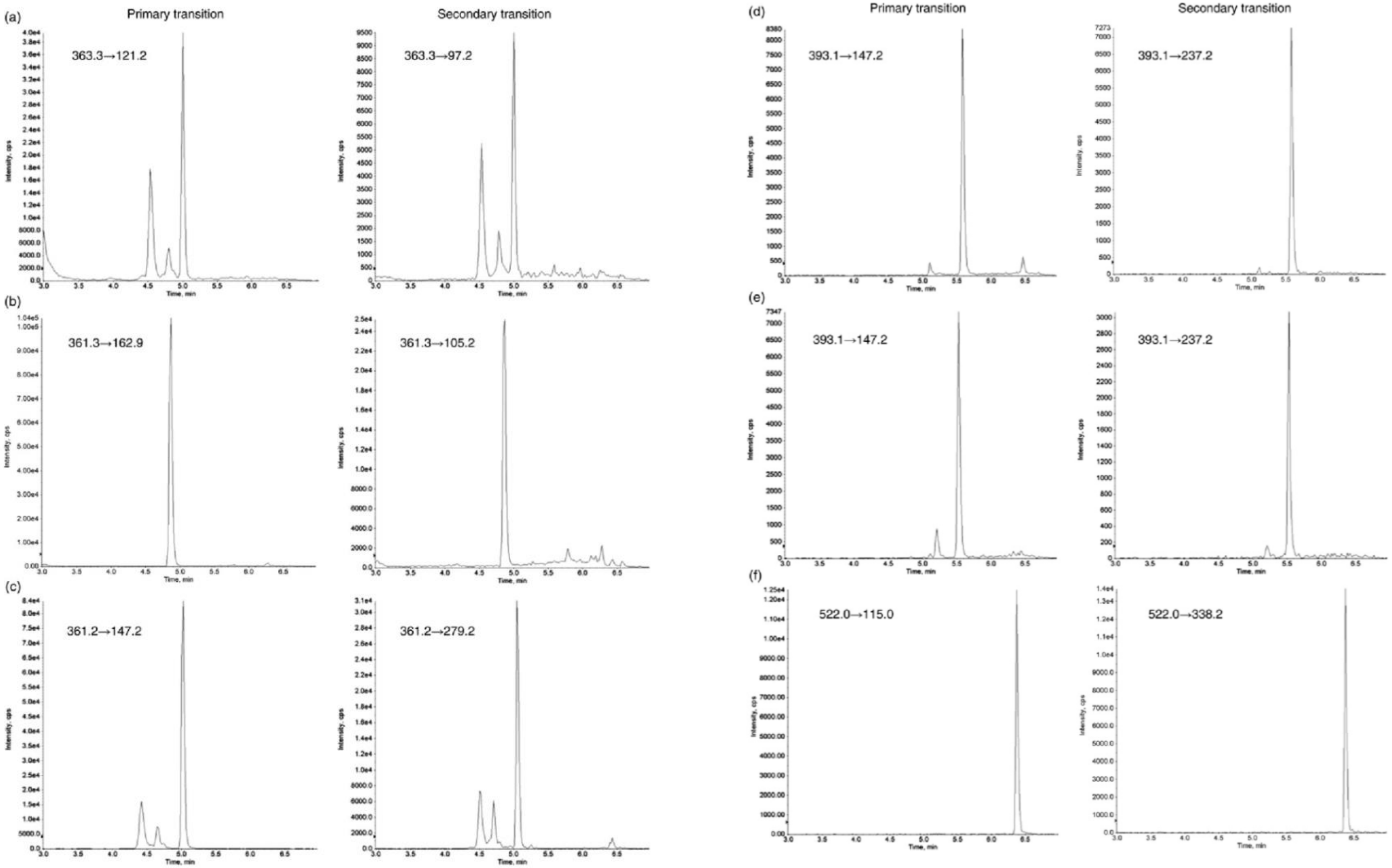


Figure 2 Liquid chromatography-tandem mass spectrometry (LC-MS/MS) chromatograms showing the primary and secondary transitions for patient samples containing (a) cortisol, (b) cortisone, (c) prednisolone, (d) dexamethasone, (e) betamethasone and (f) a urine sample spiked with beclomethasone dipropionate (500 nmol/L)

METODICHE CROMATOGRAFICHE

VANTAGGI

MAGGIOR ACCURATEZZA DIAGNOSTICA (specificità)

INFORMAZIONI presenza contemporanea più metaboliti
presenza di steroidi esogeni
rapporto cortisolo/metaboliti
attività enzimatica endogena
significato clinico dei metaboliti

PERCHE' ANCORA FUTURO ?

SVANTAGGI

Costi più elevati

Complessità del metodo (estrazione, identificazione standard, dei cut-off, personale addestrato …)

Minore produttività analitica

Persistenza di interferenze con alcune sostanze (HPLC)

METODICHE CROMATOGRAFICHE

UN FUTURO MIGLIORE?

Miglioramento dell'accuratezza diagnostica del dosaggio di UFC nello screening CS

Riduzione della variabilità del dosaggio

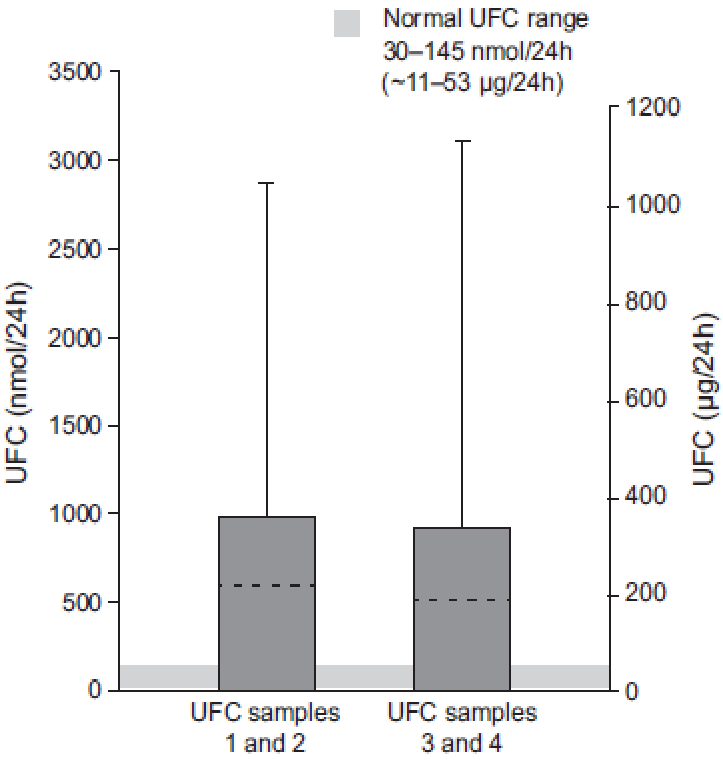
Applicabilità in condizioni cliniche in cui attualmente il dosaggio di UFC non è affidabile

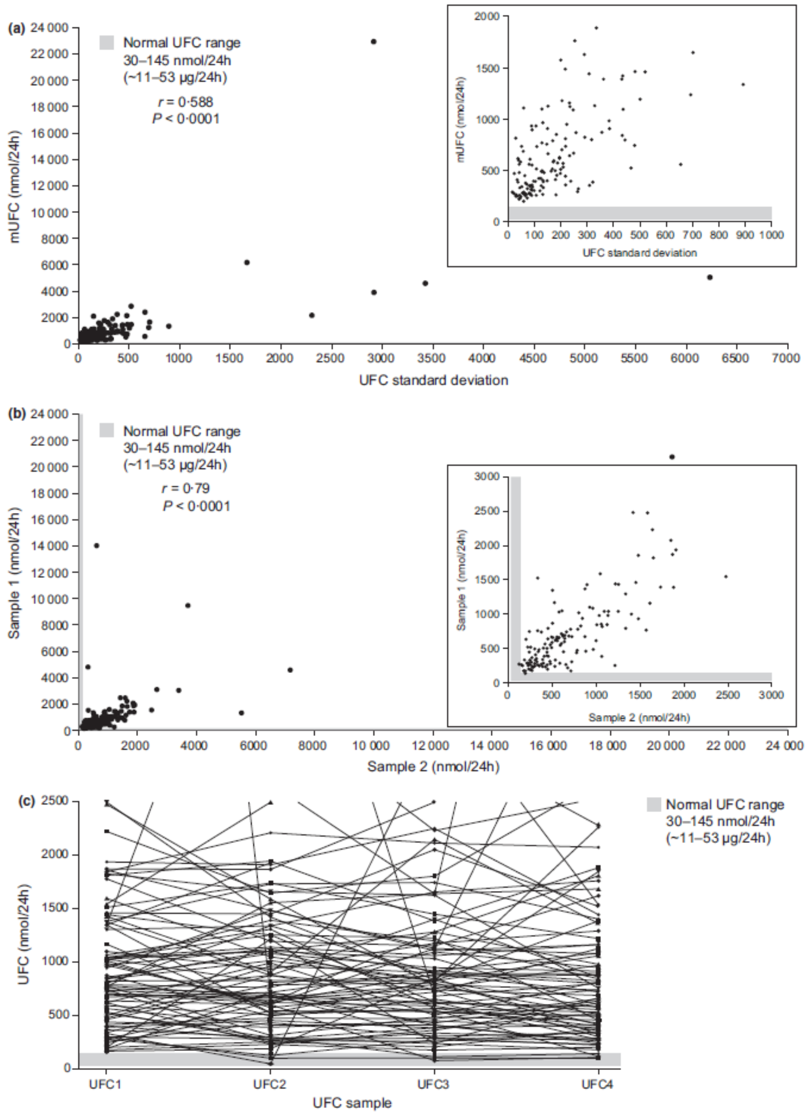
High variability in baseline urinary free cortisol values in patients with Cushing's disease

S. Petersenn*, J. Newell-Price†, J. W. Findling‡, F. Gu§, M. Maldonado¶, K. Sen**, L. R. Salgado††, A. Colao‡‡ and B. M. K. Biller§§ on behalf of the Pasireotide B2305 Study Group

Clinical Endocrinology (2014) 80, 261–269

HPLC





Concludendo…

- Il dosaggio di UFC è il passato ..ma ancora il presente nello screening della sindrome di Cushing
- Inadeguato in particolari condizioni di ipercortisolismo (mild, subclinico, ciclico…)
- I dosaggi immunometrici dimostrano un'accuratezza non ottimale
- La LC-MS/MS migliora l'accuratezza del dosaggio ma ad oggi rappresenta ancora il futuro…prossimo?
- Superiorità di LC-MS/MS in condizioni di ipercortisolismo di difficile diagnosi ??