

ABC, Napoli 2017

Il cortisolo salivare nella diagnosi
dell'ipercortisolismo: i limiti

Giovanna Mantovani

Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico

Università degli Studi di Milano

giovanna.mantovani@unimi.it

Il cortisolo salivare nella diagnosi dell'ipercortisolismo: i limiti

- **Limiti pre-analitici**
- **Limiti legati al dosaggio**
- **Limiti legati all'interpretazione e alla conseguente utilità diagnostica**

Limiti pre-analitici

- Aderenza all'orario raccomandato
- Correttezza nella modalità di raccolta:
 - Fumo
 - Cibo (liquirizia)
 - (Contaminazione con sangue) Kivlighan et al., 2004
 - Contaminazione con steroidi
- Quantità di materiale raccolto (LC-MSMS > Immunoassay)
- Device di raccolta (es. poliestere/cotone)

Limiti pre-analitici

The Relationship between Smoking Status and Cortisol Secretion

Ellena Badrick, Clemens Kirschbaum, and Meena Kumari

JCE&M 2007

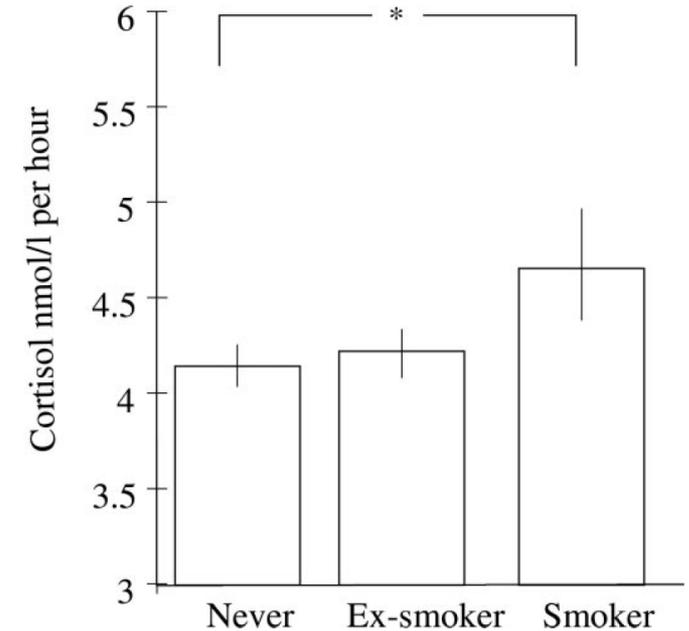
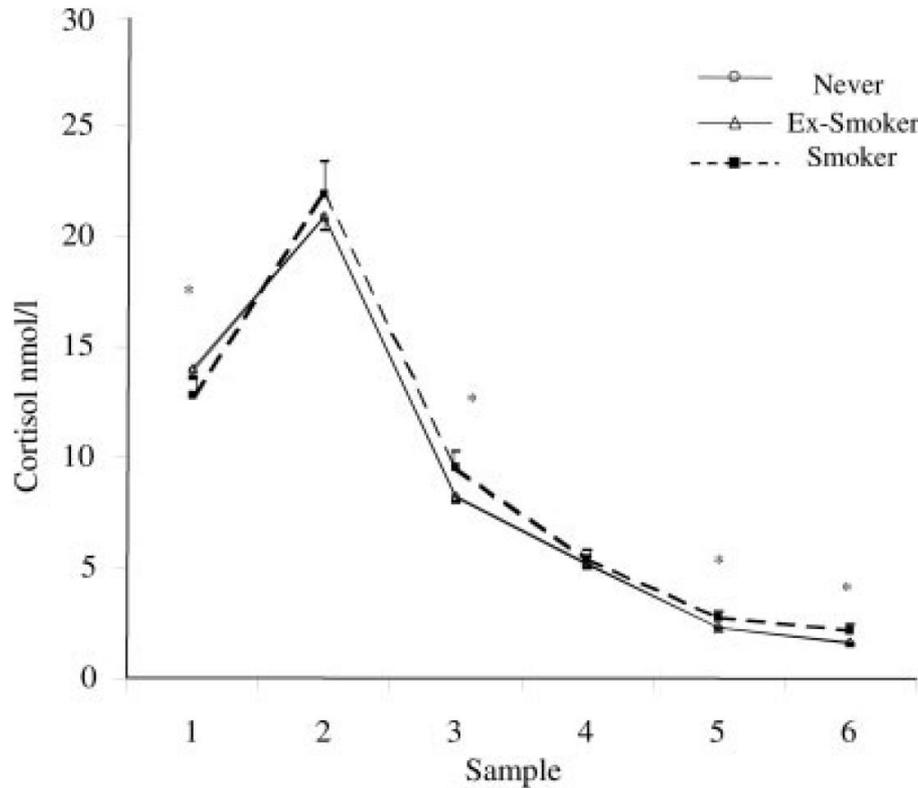
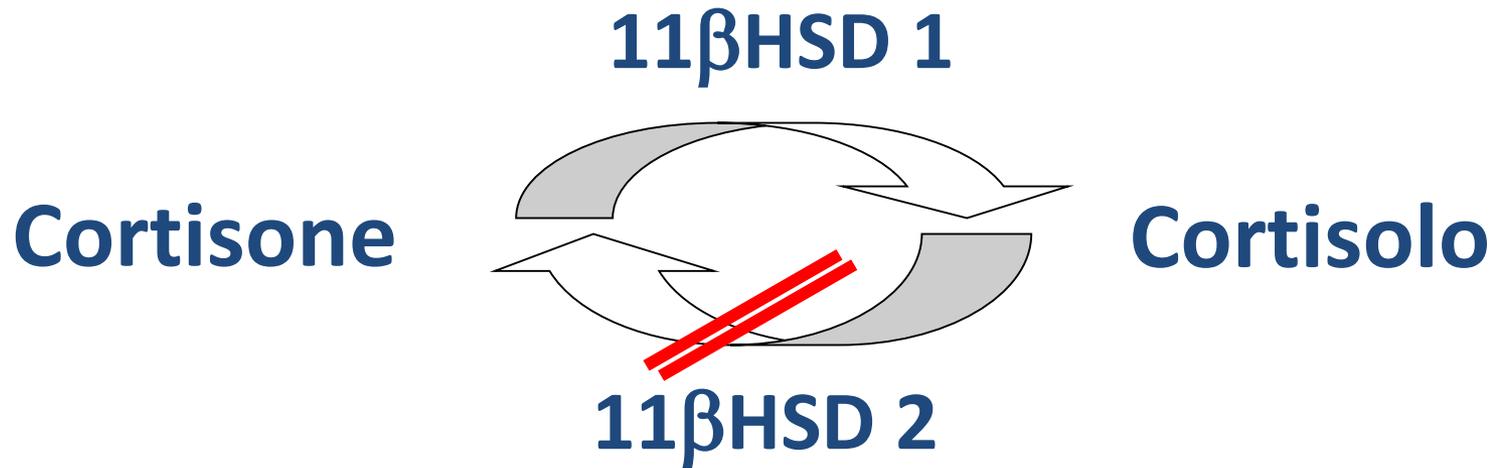


FIG. 2. Average cortisol release (adjusted means; error bars represent 95% CI) by smoking status (ANOVA, $P = 0.008$), adjusted for age, gender, and last known employment grade. *, $P = 0.002$.

Fumatori: aumento della secrezione globale di cortisolo, inclusa quella notturna

Limiti pre-analitici



Tabacco e liquirizia

Il cortisolo salivare nella diagnosi dell'ipercortisolismo: i limiti

- Limiti pre-analitici
- **Limiti legati al dosaggio**
- Limiti legati all'interpretazione e alla conseguente utilità diagnostica

Limiti legati al dosaggio

Due metodi

❑ Immunoassays : RIA – ELISA – ECLIA

- ✓ Più semplici ed economici nel setting
- ✓ Cross-reattività con altri steroidi
(11-desossicortisolo, 21-desossicortisolo, 6 β -idrossicortisolo, prednisone, prednisolone, corticosterone)

❑ Liquid chromatography – mass spectrometry (LC-MS)

- ✓ > sensibilità (10-100 volte)
- ✓ > specificità
- ✓ > costo (soprattutto iniziale)

Limiti legati ai dosaggi: confronto

Table 2 Diagnostic performance of salivary cortisol (late-night sample) for Cushing's syndrome

Assay	Cut-off for Cushing's syndrome (nmol/l) ^a	Sensitivity (%)	Specificity (%)
RIA	3.5–11.0	92–100	77–100
FPIA	15.2	93	100
ECLIA	8.3–9.7	100	89–97
LC–MS/MS	2.8	93	91

Table 1 Pertinent comparisons of salivary cortisol assays

	Direct immunoassay ELISA (Manual)	Direct immunoassay ELCIA/ELFIA (Automated)	LC–MS/MS
Sample volume	50 µl ^a	40 µl ^a	100–250 µl (extraction)
Analytic sensitivity	0.4 nmol/l	1.0 nmol/l	0.3–2.0 nmol/l ^b
Specificity	Can crossreact with cortisone and some synthetic steroids	Can crossreact with cortisone and some synthetic steroids	Minimal crossreactivities—can report cortisone and synthetic steroids
Cost	Minimal	Minimal	High start-up costs
Degree of difficulty	Minimal	Minimal	Significant labor cost and expertise

Limiti legati al dosaggio

In entrambi i casi:

Mancanza di intervalli di riferimento attendibili (range di riferimento che non tengono conto di: anziani, DM2, obesi, pseudo-Cushing)

Table 1. Assay techniques, reference ranges and diagnostic performance in studies of salivary cortisol

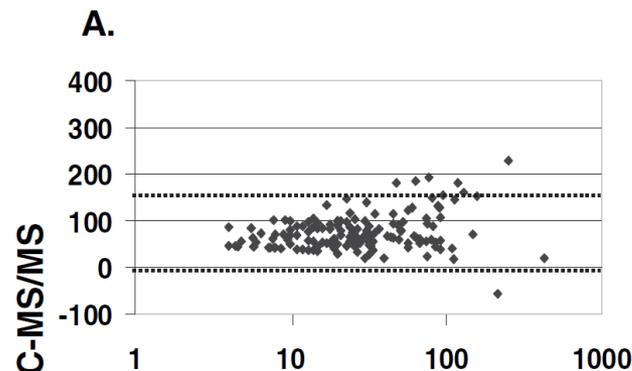
Author (year)	Assay	Analytical sensitivity (nM)	Upper limit of normal (nM)	Included pseudo-Cushing's	Diagnostic sensitivity (%)	Specificity (%)
Raff (1998) ³³	RIA	0.4	3.6	Yes	92	NR
Papanicolau (2002) ³⁴	RIA	2.2–5.8	15.2	Yes	93	100
Putignano (2003) ¹³	RIA	1.4	9.7	Yes	92.7	93.1
Yaneva (2004) ³⁵	RIA	1.66	5.5	No	100	96
Viardot (2005) ⁷²	RIA	0.8	6.1	Yes	100	100
Baid (2007) ^{*,†,11}	RIA	1.4	4.7	Yes	NR	86
	LC-MSMS	0.11	2.8		NR	94 [‡] /92 [§]
Nunes (2009) ²⁵	RIA	2.0	12 [¶]	No	100	100
			8 ^{**}		90	91.8
			4.8 ^{††}		77	69.1
Zerikly (2010) ^{*,24}	LC-MSMS	NR	2.76	Yes	93	91
Beko (2010)	ECLIA	NR	9.7	Yes	100	88
	RIA	NR	8.0		100	71
Leon-Justel (2011) ⁷³	ECLIA	NR	10	Yes	96.8	87
Erickson (2012) ^{*,36}	LC-MSMS	0.11	2.8	Yes	74.5	90.1

Limiti legati al dosaggio

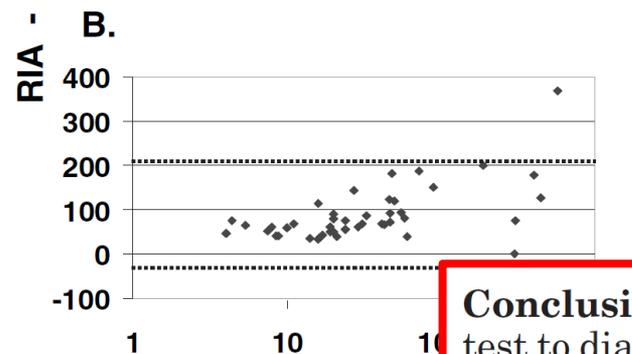
Radioimmunoassay and Tandem Mass Spectrometry Measurement of Bedtime Salivary Cortisol Levels: A Comparison of Assays to Establish Hypercortisolism

JCE&M 2007

Smita K. Baid, Ninet Sinaii, Matt Wade, Domenica Rubino, and Lynnette K. Nieman



Soggetti obesi



Volontari sani

Important rate of abnormal LNSC in non-Cushing subjects by two different commercial assays, with laboratory-provided normative ranges

LC-MS/MS Salivary

Bland-Altman plots

Conclusion: Salivary cortisol levels should not be used as the sole test to diagnose Cushing's syndrome if laboratory-provided reference ranges are used for diagnostic interpretation. (*J Clin Endocrinol Metab* 92: 3102–3107, 2007)

Limiti legati al dosaggio

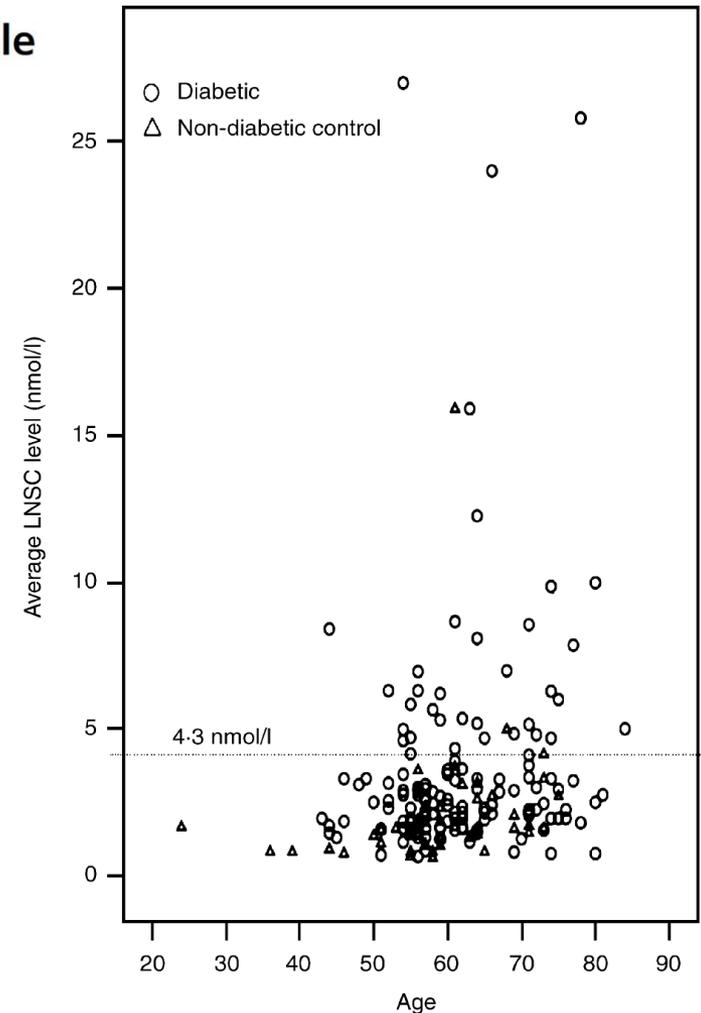
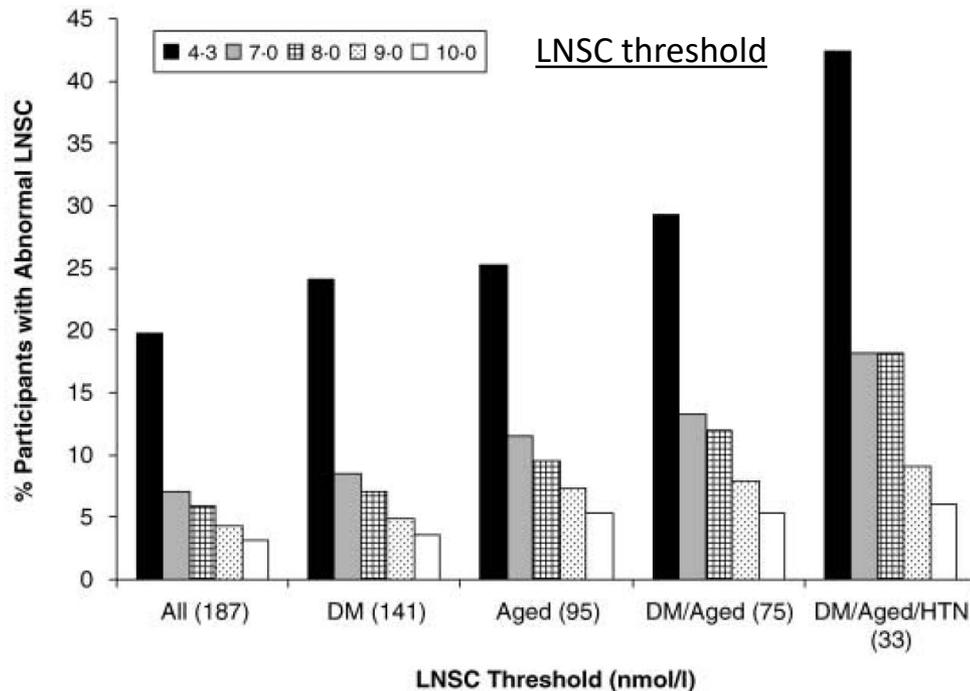
Clinical Endocrinology (2005) 63, 642–649

Assay: EIA

ORIGINAL ARTICLE

Elevated late-night salivary cortisol levels in elderly male type 2 diabetic veterans

Hau Liu*†, Dena M. Bravata†, Josel Cabaccan*, Hershel Raff‡ and Elisabeth Ryzen*§



Condizioni parafisiologiche: la gravidanza

European Journal of Endocrinology (2013) 168 315–321

ISSN 0804-4643

CLINICAL STUDY

Usefulness of salivary cortisol in the diagnosis of hypercortisolism: comparison with serum and urinary cortisol

Assay: RIA

Luca Manetti, Giuseppe Rossi¹, Lucia Grasso, Valentina Raffaelli, Ilaria Scattina, Simone Del Sarto¹, Mirco Cosottini², Aldo Iannelli², Maurizio Gasperi³, Fausto Bogazzi and Enio Martino

	CD untr (<i>n</i> =27)	Controls (<i>n</i> =89)	OCP (<i>n</i> =21)	Pregnancy (<i>n</i> =18)
MSC ≤2.77 ng/ml	0	87	20	15
MSC >2.77 ng/ml	27	2	1	3
Sensitivity (95% CI)	100% (87.2–100)	97.7% (92.1–99.7)	95.2 (76.2–99.9)	83.3% (58.6–96.4)
SC _{LDDST} ≤1.22 ng/ml	1	87	19	ND
SC _{LDDST} >1.22 ng/ml	26	2	2	ND
Sensitivity (95% CI)	96.3% (81.0–99.9)	97.7% (92.1–99.7)	90.5% (69.6–98.8)	
SeC _{LDDST} ≤18 ng/ml	0	87	13	ND
SeC _{LDDST} >18 ng/ml	27	2	8	ND
Sensitivity (95% CI)	100% (87.2–100)	97.7% (92.1–99.7)	61.9% (38.4–81.9)	
MSeC ≤18 ng/ml	0	ND	ND	ND
MSeC >18 ng/ml	27	ND	ND	ND
Sensitivity (95% CI)	100% (87.2–100)	ND		
UFC ≤346 µg/24 h	2	87	21	ND
UFC >346 µg/24 h	23	2	0	ND
Sensitivity (95% CI)	92.6% (75.7–99.1)	97.7% (92.1–99.7)	100% (83.9–100)	

Limiti legati al dosaggio

Summary and critical appraisal of laboratory aspects

The interpretation of salivary cortisol levels relies on rigorous standardization of sampling equipment, sampling protocols and assay technology. With such a wide variety of practices in the literature, it is not possible to state the 'correct' laboratory techniques to follow. At our own institution, we have implemented

Il cortisolo salivare nella diagnosi dell'ipercortisolismo: i limiti

- Limiti pre-analitici
- Limiti legati al dosaggio
- **Limiti legati all'interpretazione e alla conseguente utilità diagnostica**

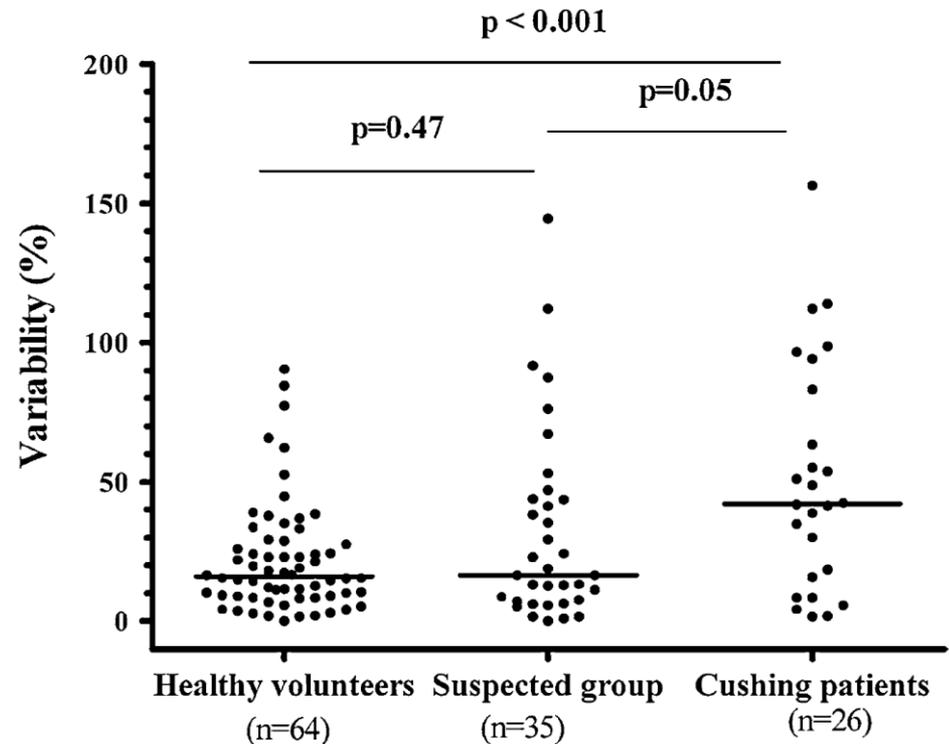
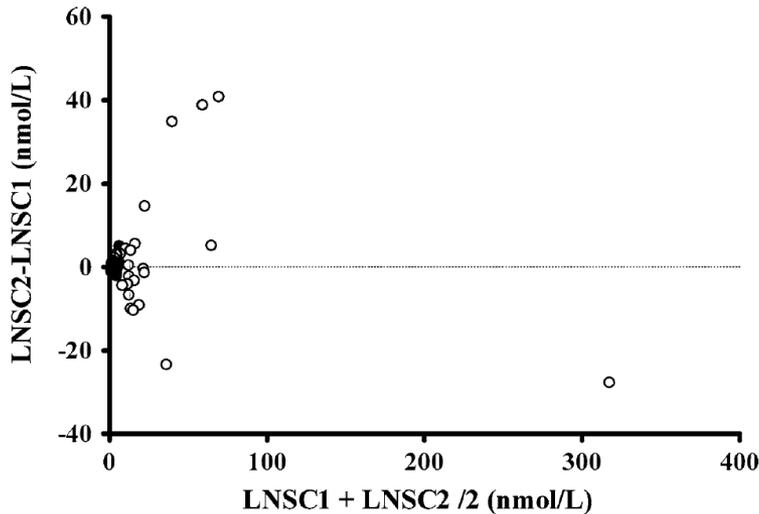
Limiti in diagnostica: la riproducibilità

Endocrine (2012) 41:487–493
DOI 10.1007/s12020-012-9597-z

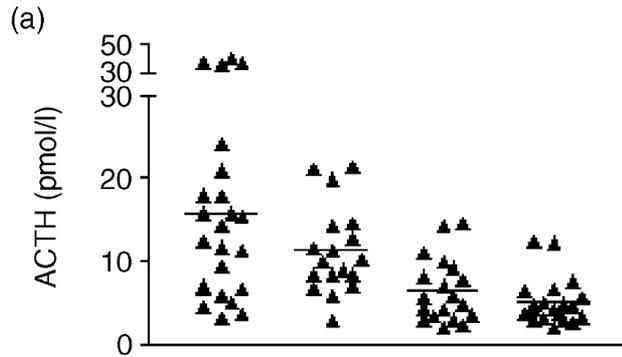
ORIGINAL ARTICLE

Assay: ECLIA

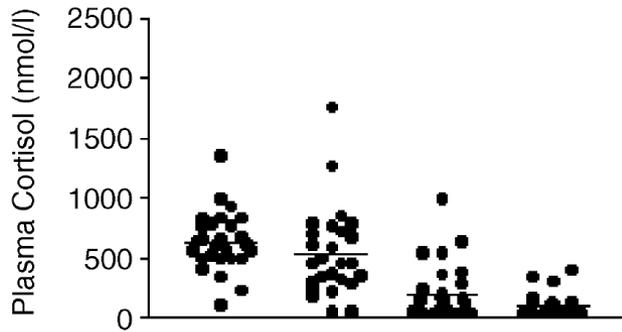
Reproducibility and performance of one or two samples of salivary cortisol in the diagnosis of Cushing's syndrome using an automated immunoassay system



Limiti in diagnostica: il Cushing

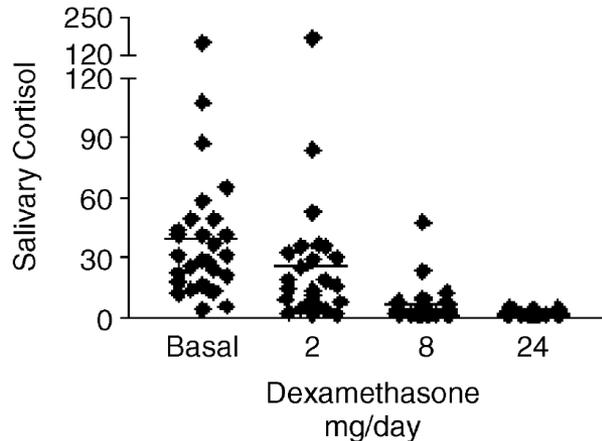


Malattia di Cushing N=28



La soppressione del cortisolo salivare dopo desametasone 8 mg è > rispetto a quella del cortisolo plasmatico

Cut-off standard 50% ➡ 65%



Limiti in diagnostica: il Cushing

Clin Endocrinol 2012

Late-night salivary cortisol for diagnosis of Cushing's syndrome by liquid chromatography/tandem mass spectrometry assay

Dana Erickson*, Ravinder J. Singht†, Airani Sathananthan§, Adrian Vella* and Sandra C. Bryant‡

Assay: LS-MS/MS

Table 3. Sensitivity and specificity of LNSC and UFC for Cushing's syndrome

	Test abnormal/CS	Sensitivity % (CI)	Test negative/no. CS	Specificity % (CI)
LNSC normal value of <2.8 nmol/l				
LNSC – total population	35/47	74.5 (60.5, 84.7)	182/202	90.1 (85.2, 93.5)
LNSC – obese population	20/28	71.4 (52.9, 84.7)	124/135	91.8 (86.0, 95.4)
LNSC – nonobese population	15/19	83.3 (56.7, 94.5)	58/67	86.6 (71.3, 89.4)
Optimal value calculated per ROC for LNSC <2.1 nmol/l				
LNSC	41/47	83.0 (69.9, 91.1)	158/202	84.2 (78.5, 88.5)
24-h UFC normal value <124 nmol/l				
24-h UFC	39/45	86.7 (73.8, 93.7)	136/158	86.1 (79.8, 90.6)
LNSC ≥ 2.8 or 24 h UFC abnormal	43/47	91.5 (80.1, 96.6)	162/202	80.2 (74.2, 85.1)
LNSC ≥ 2.1 or 24 h UFC abnormal	45/47	95.7 (85.8, 98.9)	150/202	74.3 (67.8, 79.8)

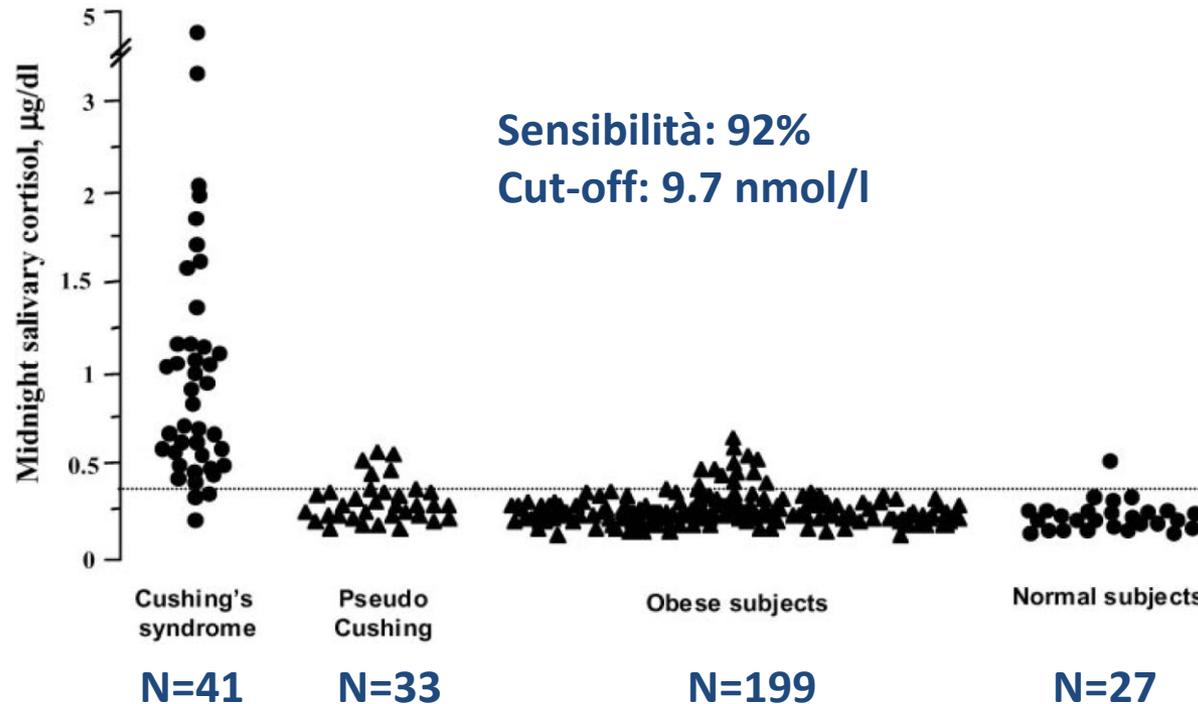
Limiti in diagnostica: il Cushing

Midnight Salivary Cortisol *Versus* Urinary Free and Midnight Serum Cortisol as Screening Tests for Cushing's Syndrome

JCE&M 2003

Assay: RIA

PIETRO PUTIGNANO, PAOLA TOJA, ANTONELLA DUBINI, FRANCESCA PECORI GIRALDI, SALVATORE MARIA CORSELLO, AND FRANCESCO CAVAGNINI



Limiti in diagnostica: il Cushing «mild»

European Journal of Endocrinology (2007) 157 725–731

ISSN 0804-4643

CLINICAL STUDY

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}

11 pz con s. di Cushing mild

Né una determinazione di CLU nella norma, né una di LNSC escludono la diagnosi

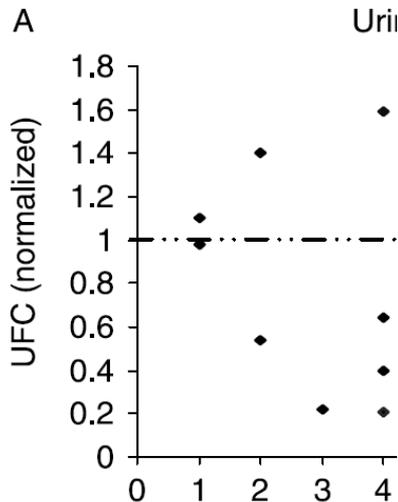
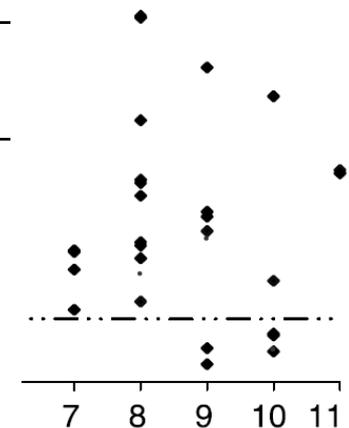


Table 2 Percentage of positive results with salivary cortisol and urine free cortisol in 11 patients with surgically proven Cushing's syndrome

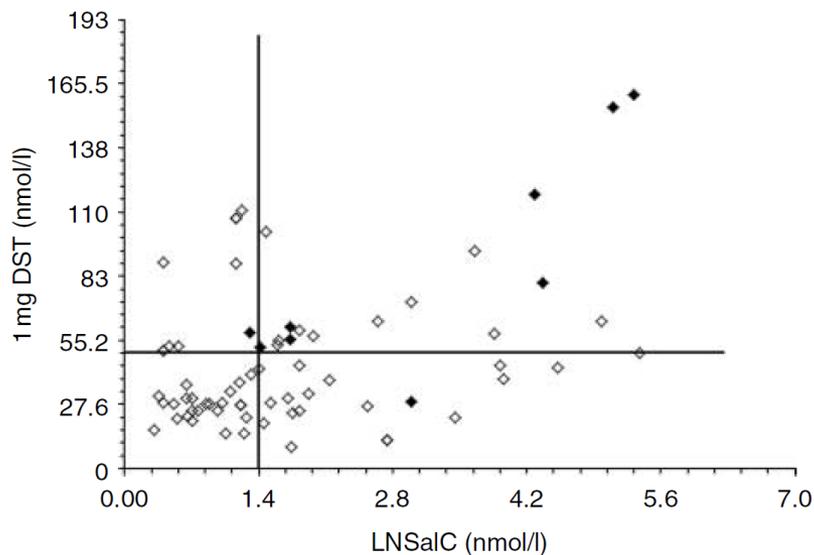
Patient no.	% Abnormal urine free cortisol	% Abnormal nocturnal salivary cortisol
1	50	78
2	50	29
3	0	14
4	25	100
5	0	100
6	0	100
7	0	100
8	0	100
9	0	71
10	66	33
11	0	100



Limiti in diagnostica: l'incidentaloma surrenalico e il Cushing subclinico

Rispetto al test di Nugent: sensibilità paragonabile ed elevata ma < specificità

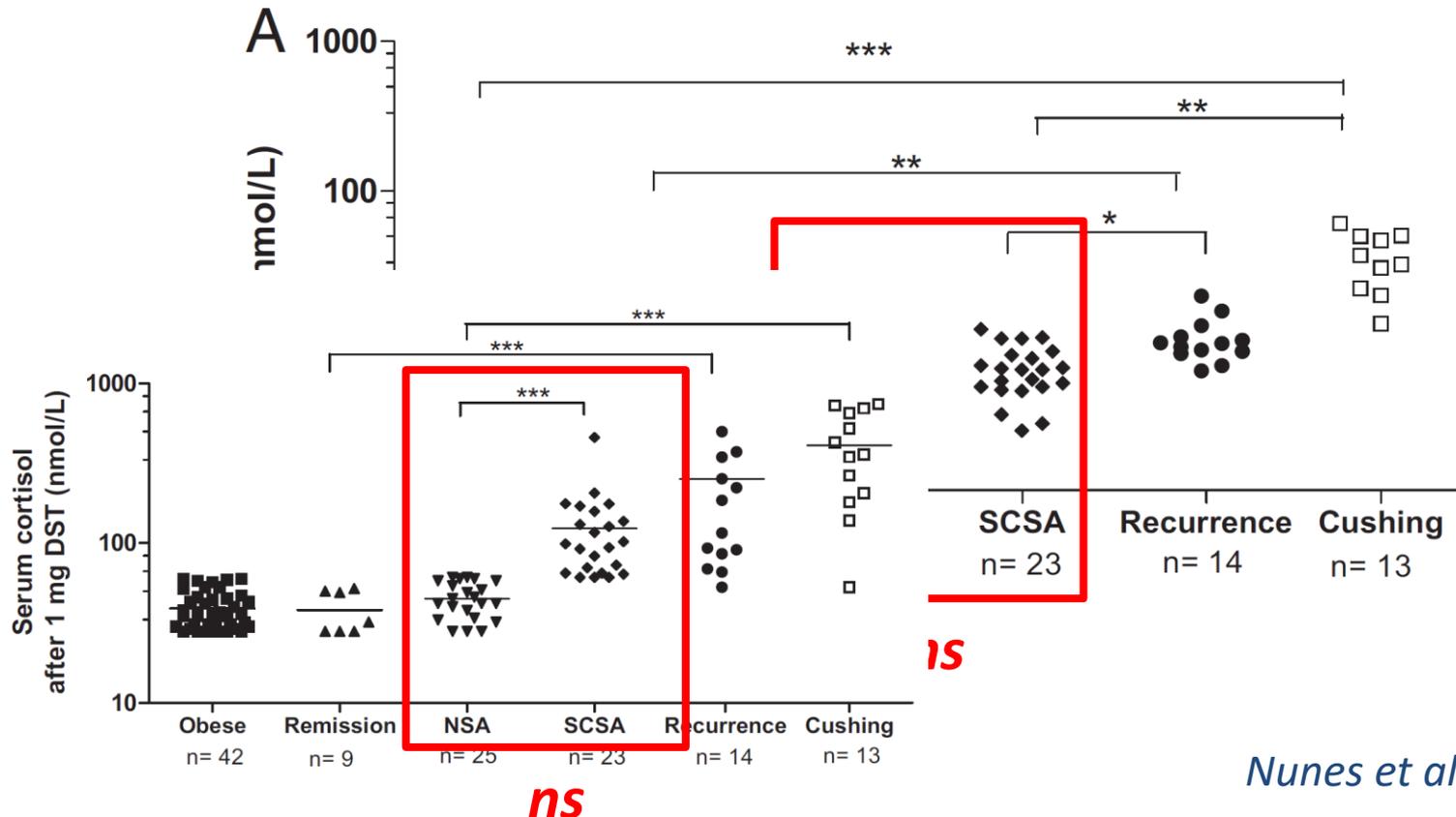
<i>Raff, Pituitary 2012</i>	Cutoff (nmol/l)	Sensitivity (%)	Specificity %
2300 h serum	115	97	63
0800 h serum after dex	94	97	88
2300 h saliva	4.8	97	69
0800 h saliva after dex	3.7	97	83



Il LNSC, solo combinato con il test di Nugent, è utile per predire le comorbidità legate all'ipercortisolismo subclinico (ipertensione, T2DM e osteoporosi)

Limiti in diagnostica: l'incidentaloma surrenalico e il Cushing subclinico

Il cortisolo salivare notturno, al contrario del test di Nugent, non discrimina gli adenomi surrealici non secernenti da quelli associati ad ipercortisolismo subclinico



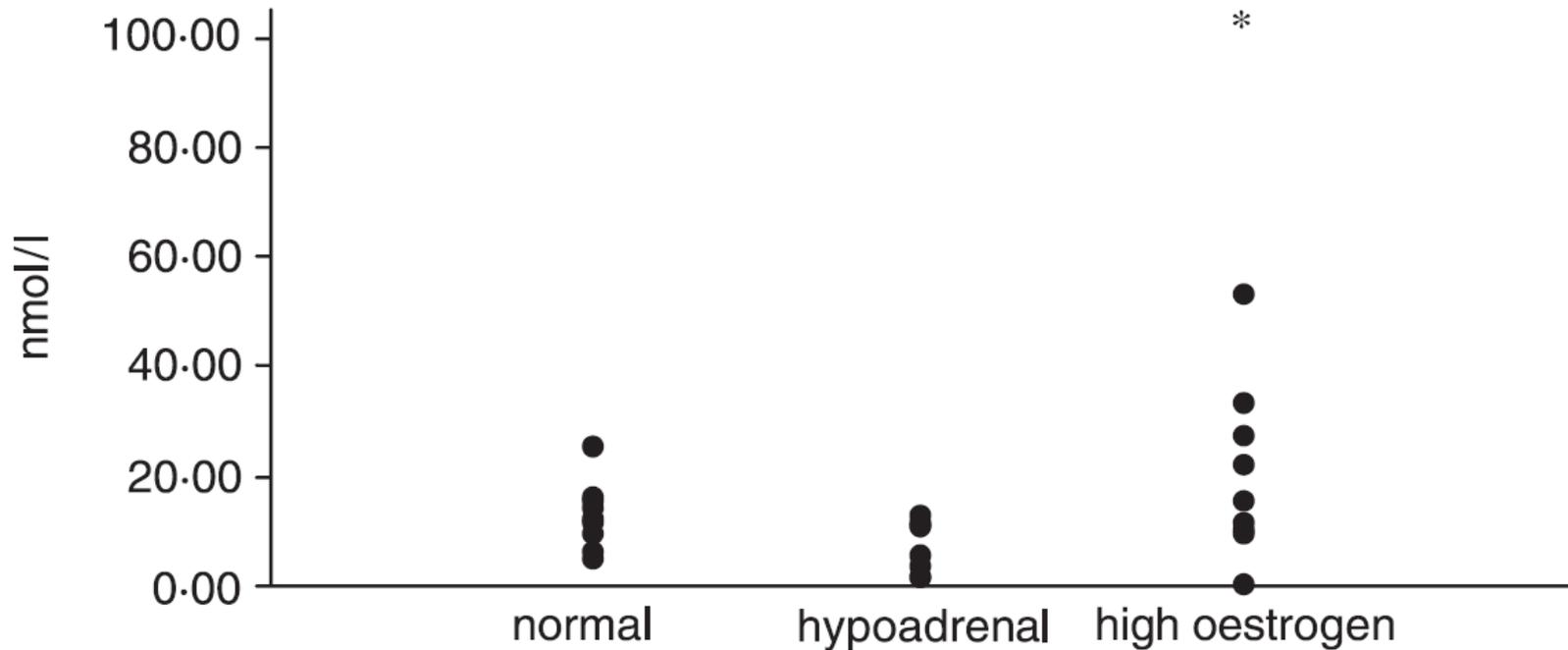
.. e nell'iposurrenalismo centrale ?

ORIGINAL ARTICLE

Clin Endocrinol 2006

Low-dose ACTH (1 μ g) salivary test: a potential alternative to the classical blood test

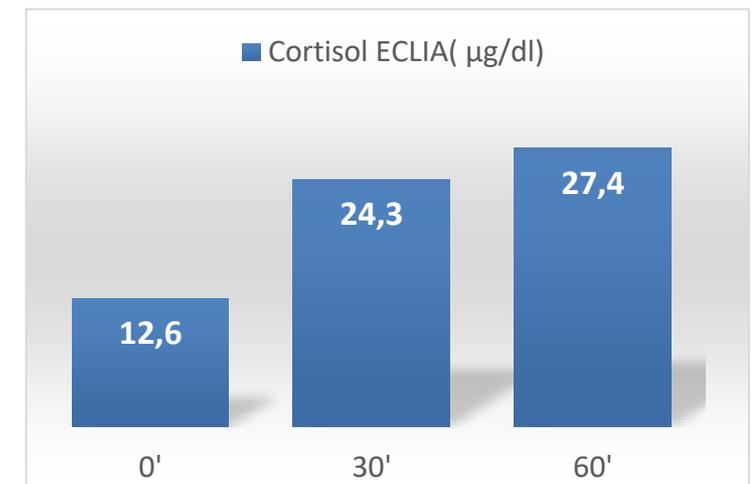
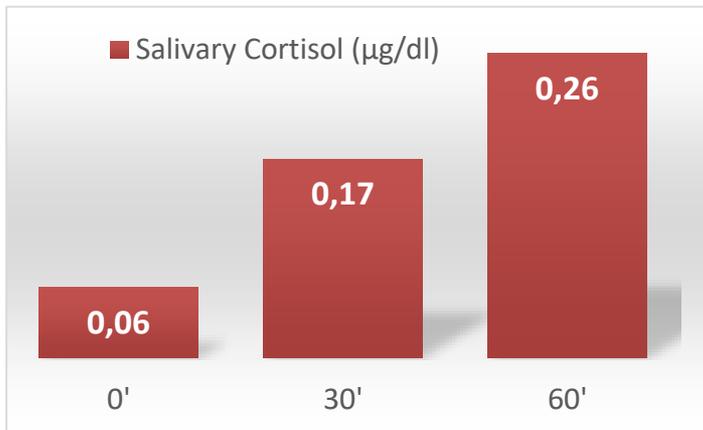
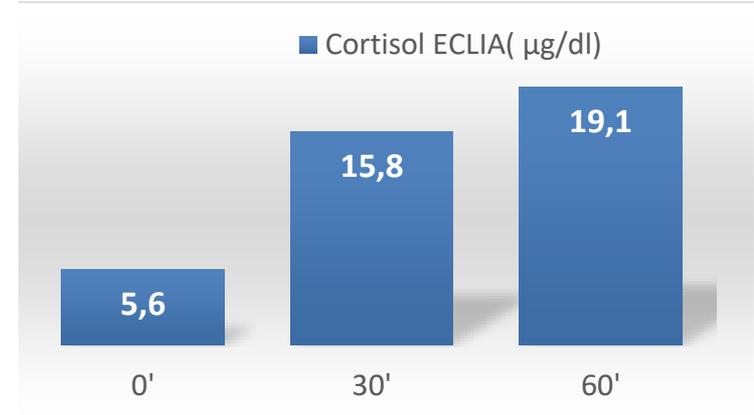
Assay: RIA



NESSUNA UTILITA' IN CONDIZIONI BASALI !!

.. e nell'iposurrenalismo centrale ?

Cut-offs of basal or stimulated serum cortisol levels after standard dose ACTH test measured by ECLIA and salivary cortisol by LC-MS/MS that establish the presence of central hypoadrenalism



Cut-offs of basal or stimulated serum cortisol levels after standard dose ACTH test measured by ECLIA and salivary cortisol LC-MS/MS that exclude the presence of central hypoadrenalism

GRAZIE PER L'ATTENZIONE!



«Gruppo Ipofisi»
Unità di Endocrinologia e Malattie del Metabolismo
Fondazione IRCCS Ca' Granda Policlinico - Milano