

ABC, Napoli 2017

Il cortisolo salivare nella diagnosi  
dell'ipercortisolismo: i limiti

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# Il cortisolo salivare nella diagnosi dell'ipercortisolismo: i limiti

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

# Limiti pre-analitici

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- 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

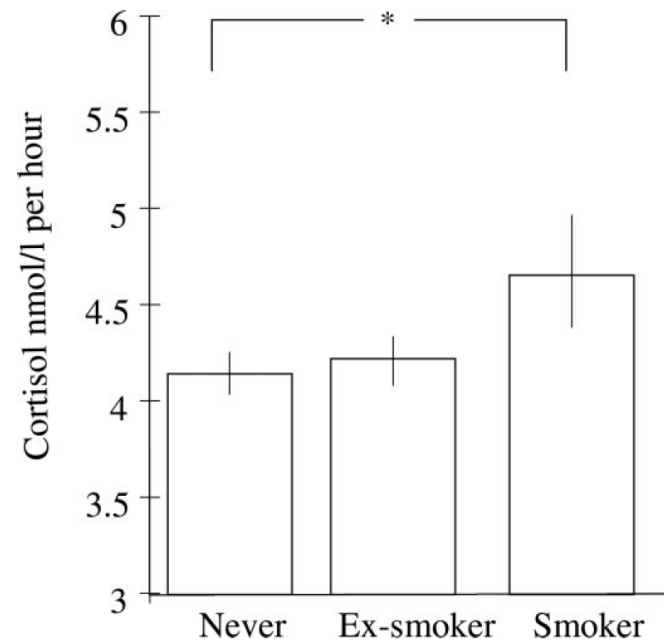
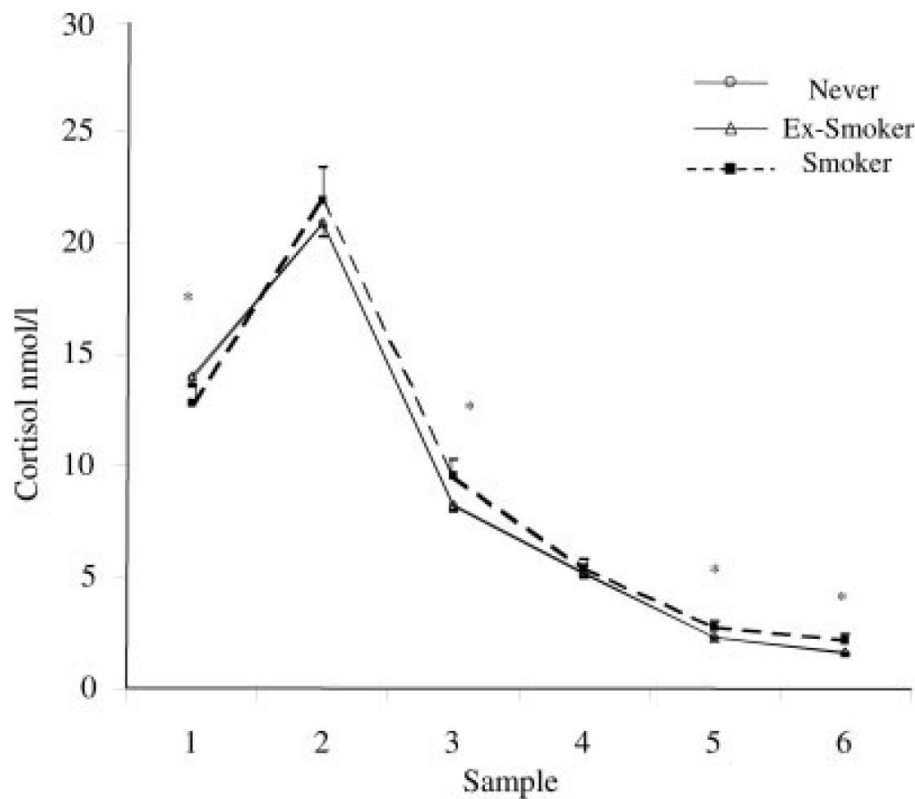
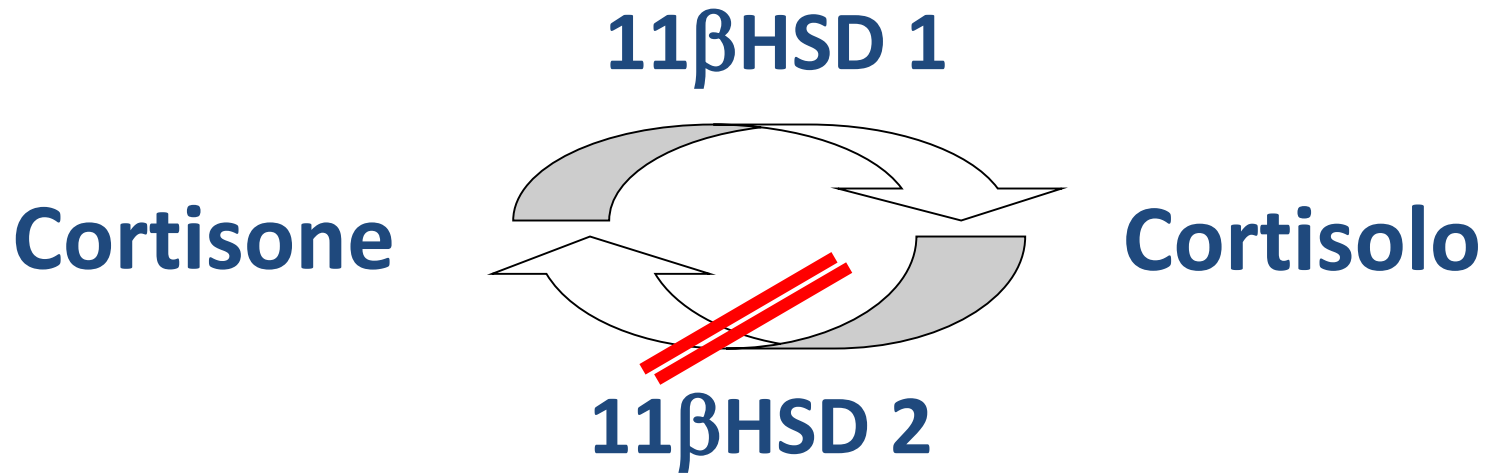


FIG. 2. Average cortisol release (adjusted means; error bars represent 95% CI) by smoking status (ANOVA,  $P = 0.008$ ), adjusted for age, gender, and last known employment grade. \*,  $P = 0.002$ .

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

# Limiti pre-analitici

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**Tabacco e liquirizia**

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# Limiti legati al dosaggio

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## Due metodi

### ❑ Immunoassays : RIA – ELISA – ECLIA

- ✓ Più semplici ed economici nel setting

- ✓ Cross-reattività con altri steroidi

(11-desossicortisolo, 21-desossicortisolo, 6 $\beta$ -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) <sup>a</sup>	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 <sup>a</sup>	40 µl <sup>a</sup>	100–250 µl (extraction)
Analytic sensitivity	0.4 nmol/l	1.0 nmol/l	0.3–2.0 nmol/l <sup>b</sup>
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

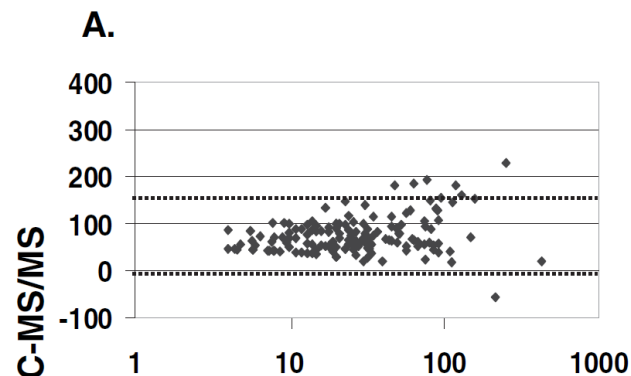
Author (year)	Assay	Analytical sensitivity (nM)	Upper limit of normal (nM)	Included pseudo-Cushing's	Diagnostic sensitivity (%)	Specificity (%)
Raff (1998) <sup>33</sup>	RIA	0.4	3.6	Yes	92	NR
Papanicolau (2002) <sup>34</sup>	RIA	2.2–5.8	15.2	Yes	93	100
Putignano (2003) <sup>13</sup>	RIA	1.4	9.7	Yes	92.7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

# Limiti legati al dosaggio

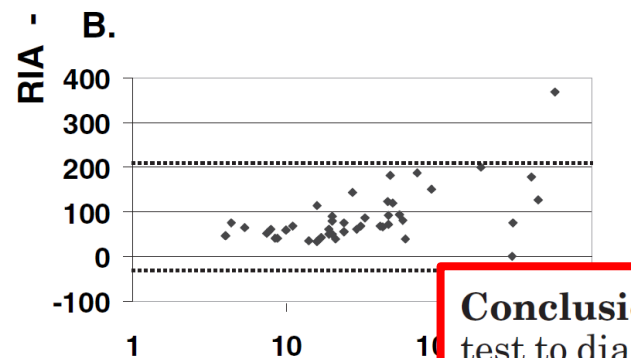
## Radioimmunoassay and Tandem Mass Spectrometry Measurement of Bedtime Salivary Cortisol Levels: A Comparison of Assays to Establish Hypercortisolism

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

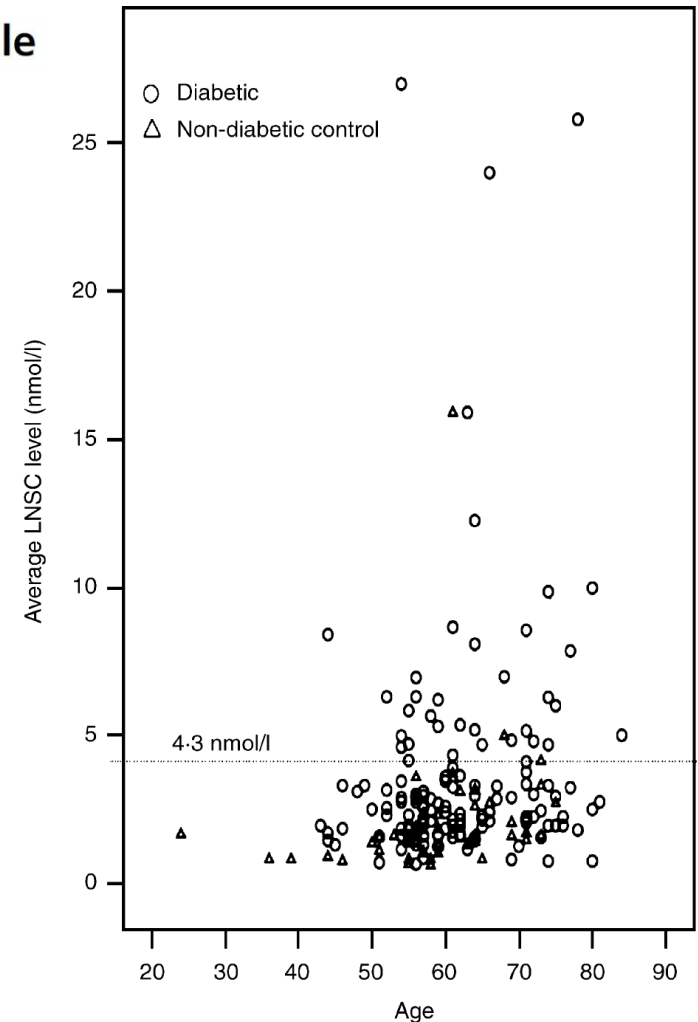
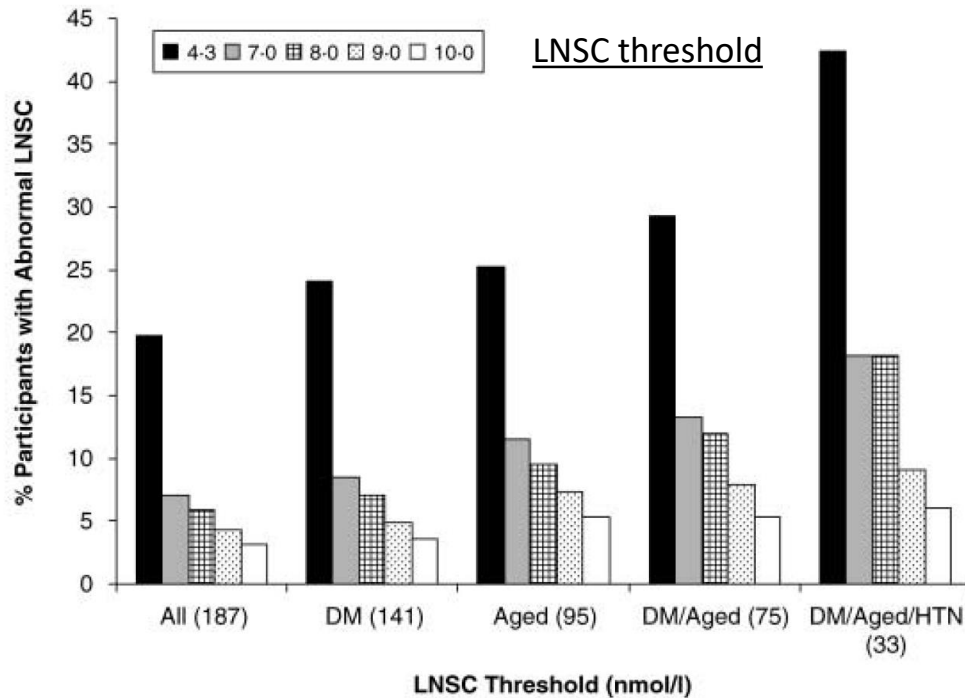
Clinical Endocrinology (2005) 63, 642–649

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 Rossi<sup>1</sup>, Lucia Grasso, Valentina Raffaelli, Ilaria Scattina, Simone Del Sarto<sup>1</sup>,  
Mirco Cosottini<sup>2</sup>, Aldo Iannelli<sup>2</sup>, Maurizio Gasperi<sup>3</sup>, Fausto Bogazzi and Enio Martino

	CD untr (n=27)	Controls (n=89)	OCP (n=21)	Pregnancy (n=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 <sub>LDDST</sub> ≤1.22 ng/ml	1	87	19	ND
SC <sub>LDDST</sub> >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 <sub>LDDST</sub> ≤18 ng/ml	0	87	13	ND
SeC <sub>LDDST</sub> >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

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## *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

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- **Limiti legati all'interpretazione e alla conseguente utilità diagnostica**

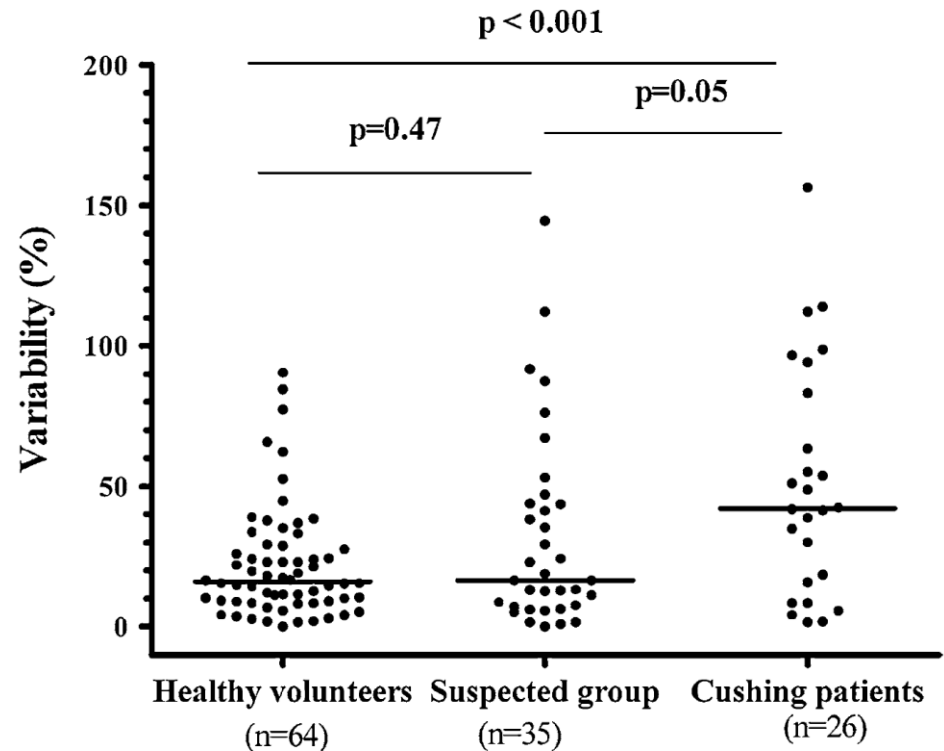
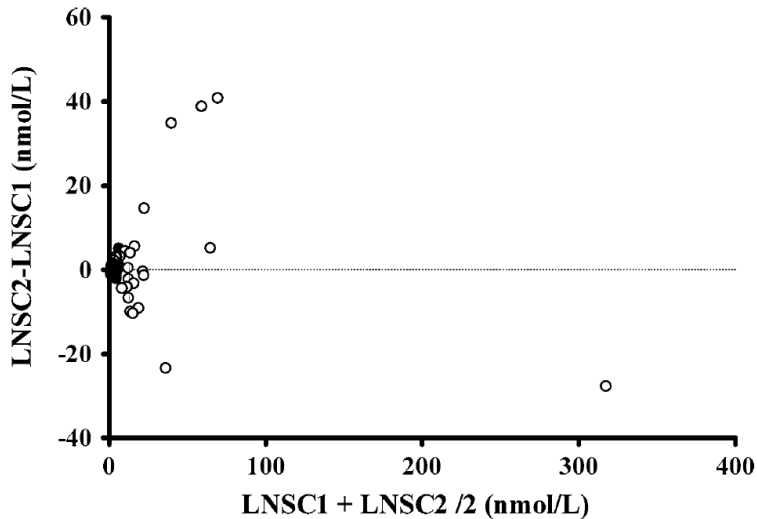
# Limiti in diagnostica: la riproducibilità

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

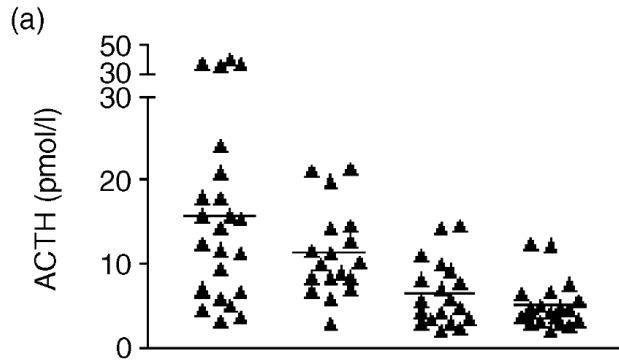
ORIGINAL ARTICLE

Assay: ECLIA

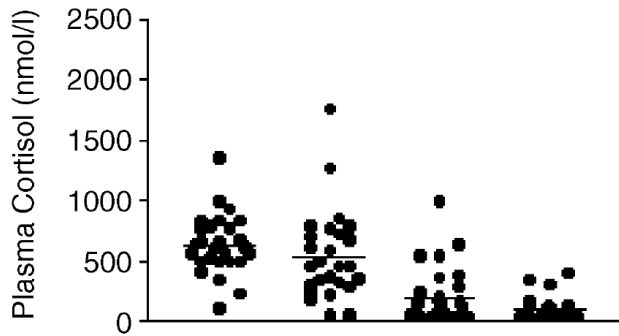
## Reproducibility and performance of one or two samples of salivary cortisol in the diagnosis of Cushing's syndrome using an automated immunoassay system



# Limiti in diagnostica: il Cushing

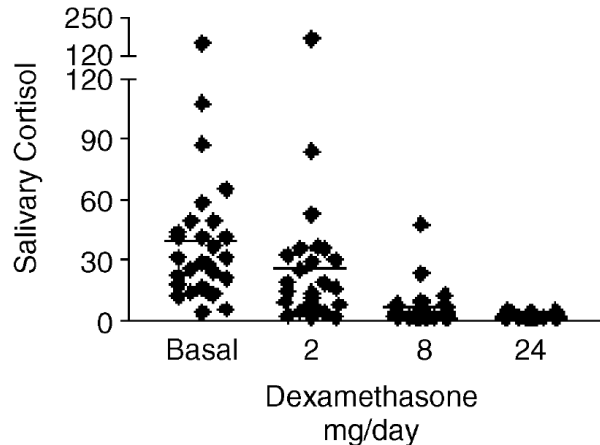


Malattia di Cushing N=28



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)
<b>LNSC normal value of &lt;2.8 nmol/l</b>				
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)
<b>Optimal value calculated per ROC for LNSC &lt;2.1 nmol/l</b>				
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)

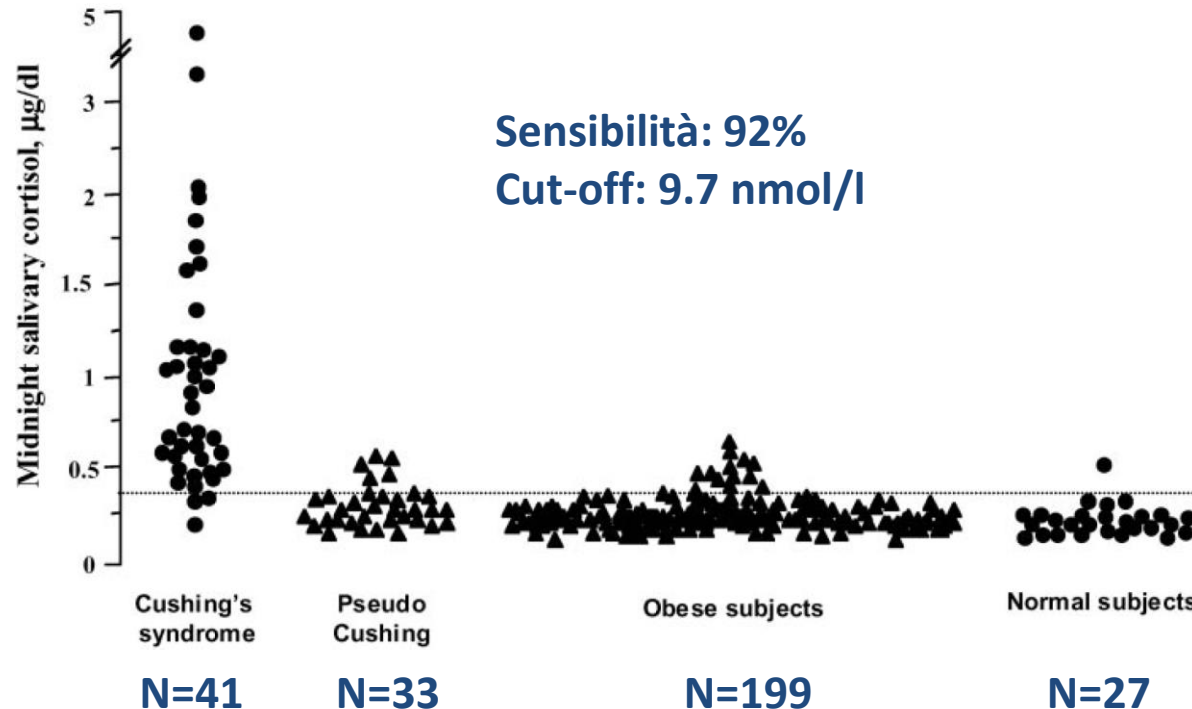
# Limiti in diagnostica: il Cushing

## 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

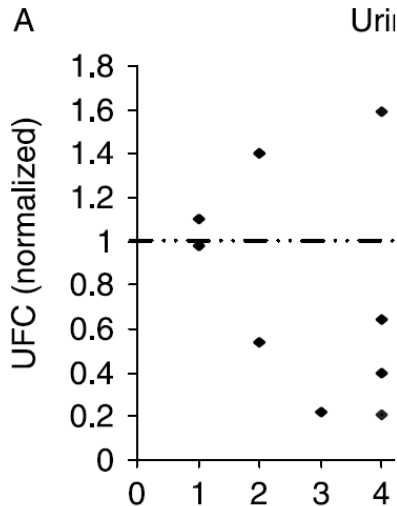
CLINICAL STUDY

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

Srividya Kidambi<sup>1,2</sup>, Hershel Raff<sup>1,2</sup> and James W Findling<sup>1,2</sup>

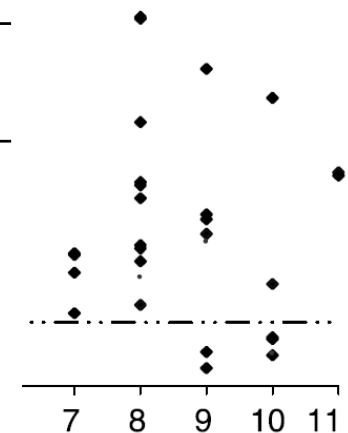
## 11 pz con s. di Cushing mild

**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

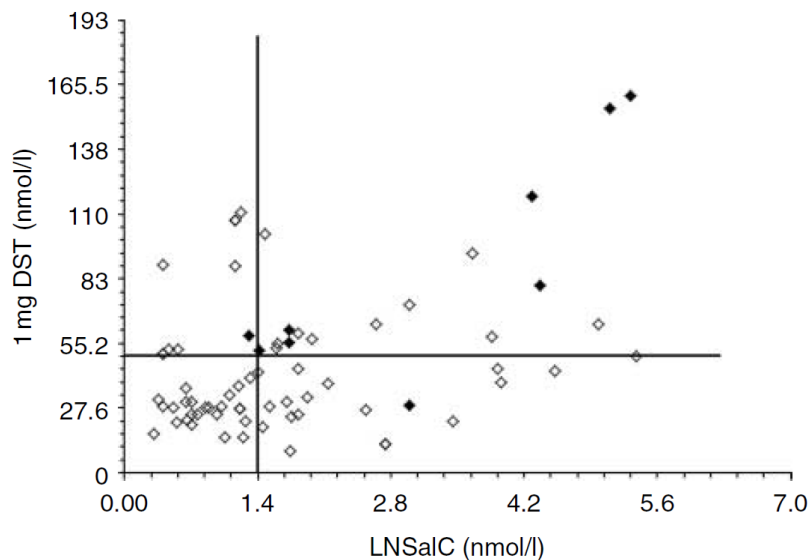
Patient no.	% Abnormal urine free cortisol	% Abnormal nocturnal salivary cortisol
1	50	78
2	50	29
3	0	14
4	25	100
5	0	100
6	0	100
7	0	100
8	0	100
9	0	71
10	66	33
11	0	100



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

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

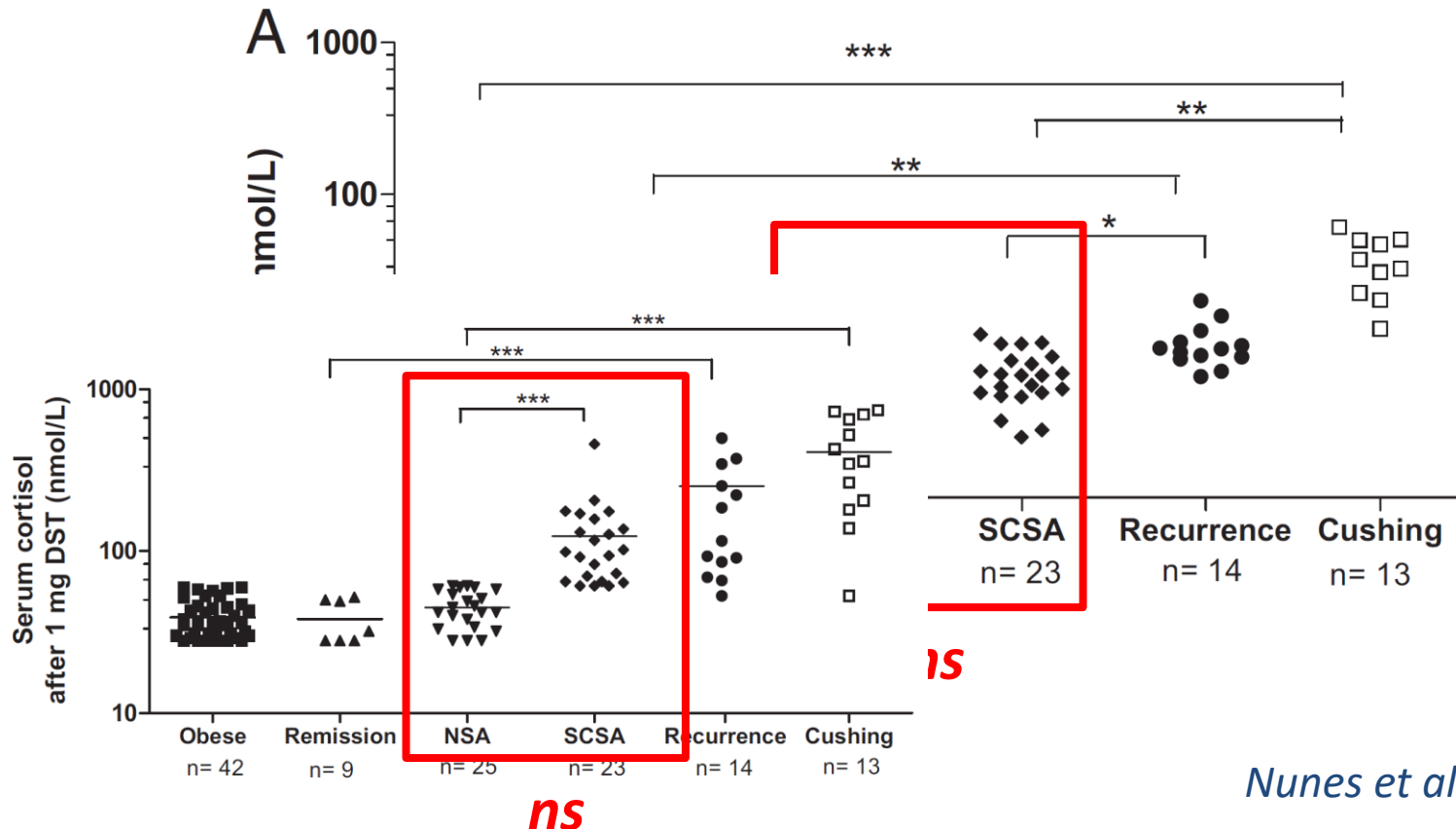
<i>Raff, Pituitary 2012</i>	Cutoff (nmol/l)	Sensitivity (%)	Specificity %
2300 h serum	115	97	63
0800 h serum after dex	94	97	88
2300 h saliva	4.8	97	69
0800 h saliva after dex	3.7	97	83



**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



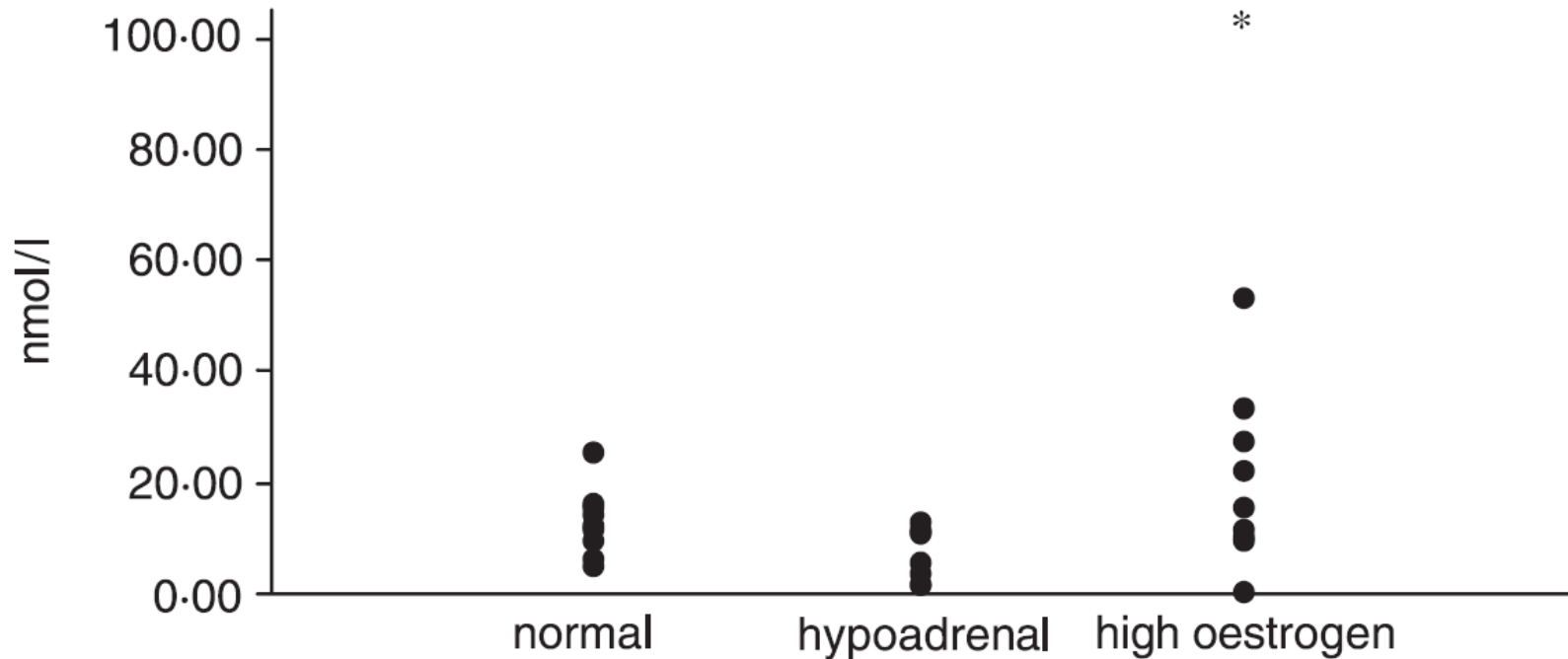
# .. e nell'iposurrenalismo centrale ?

ORIGINAL ARTICLE

*Clin Endocrinol 2006*

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

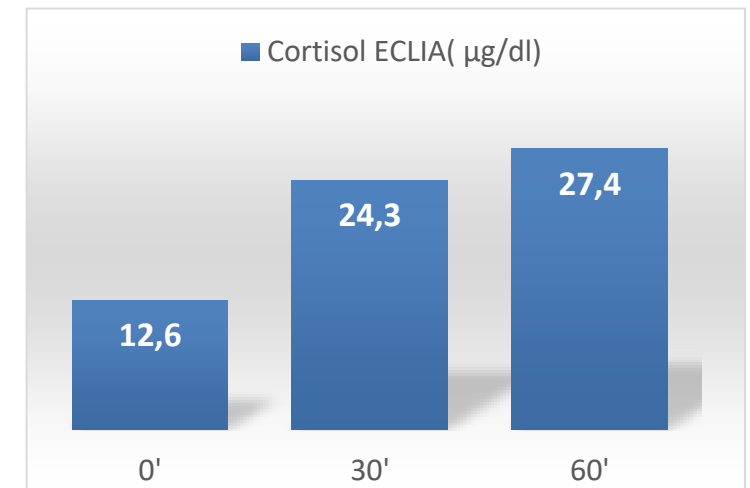
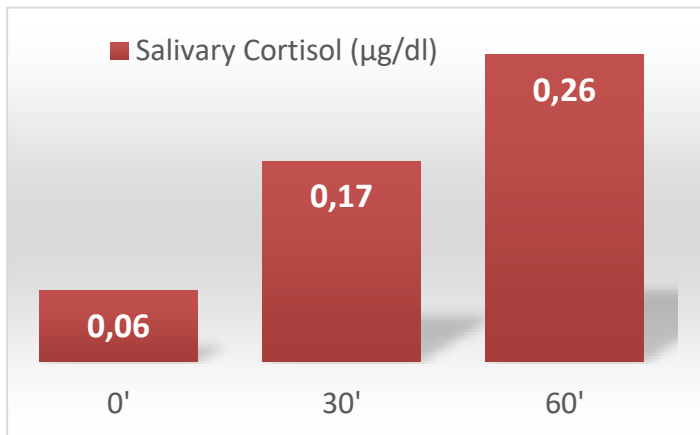
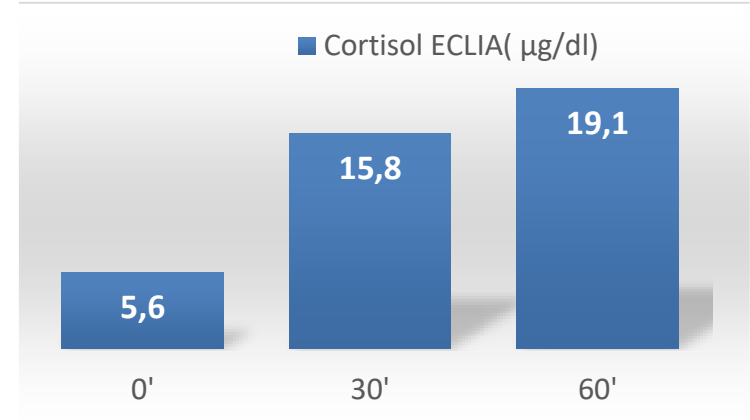
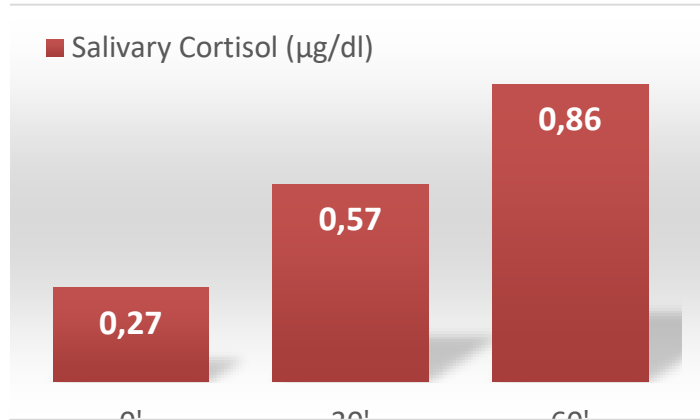
Assay: RIA



**NESSUNA UTILITA' IN CONDIZIONI BASALI !!**

# .. e nell'iposurrenalismo centrale ?

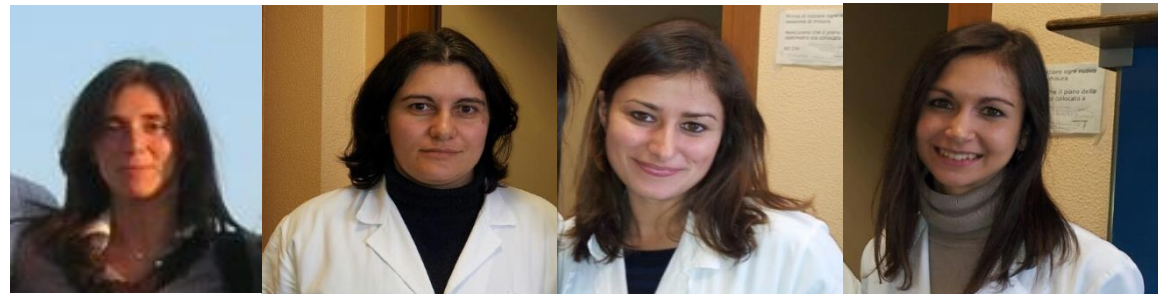
Cut-offs of basal or stimulated serum cortisol levels after standard dose ACTH test measured by ECLIA and salivary cortisol by LC-MS/MS that establish the presence of central hypoadrenalism



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!



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Fondazione IRCCS Ca' Granda Policlinico - Milano