



12° Congresso Nazionale AME 6th Joint Meeting with AACE



Il caso del cortisolo: tra certezze e pitfalls

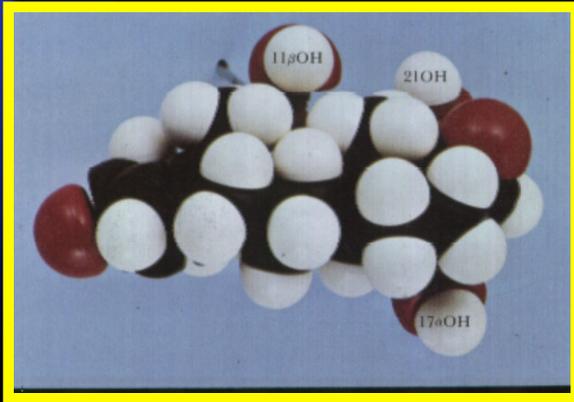
Bari, 7-10 Novembre 2013

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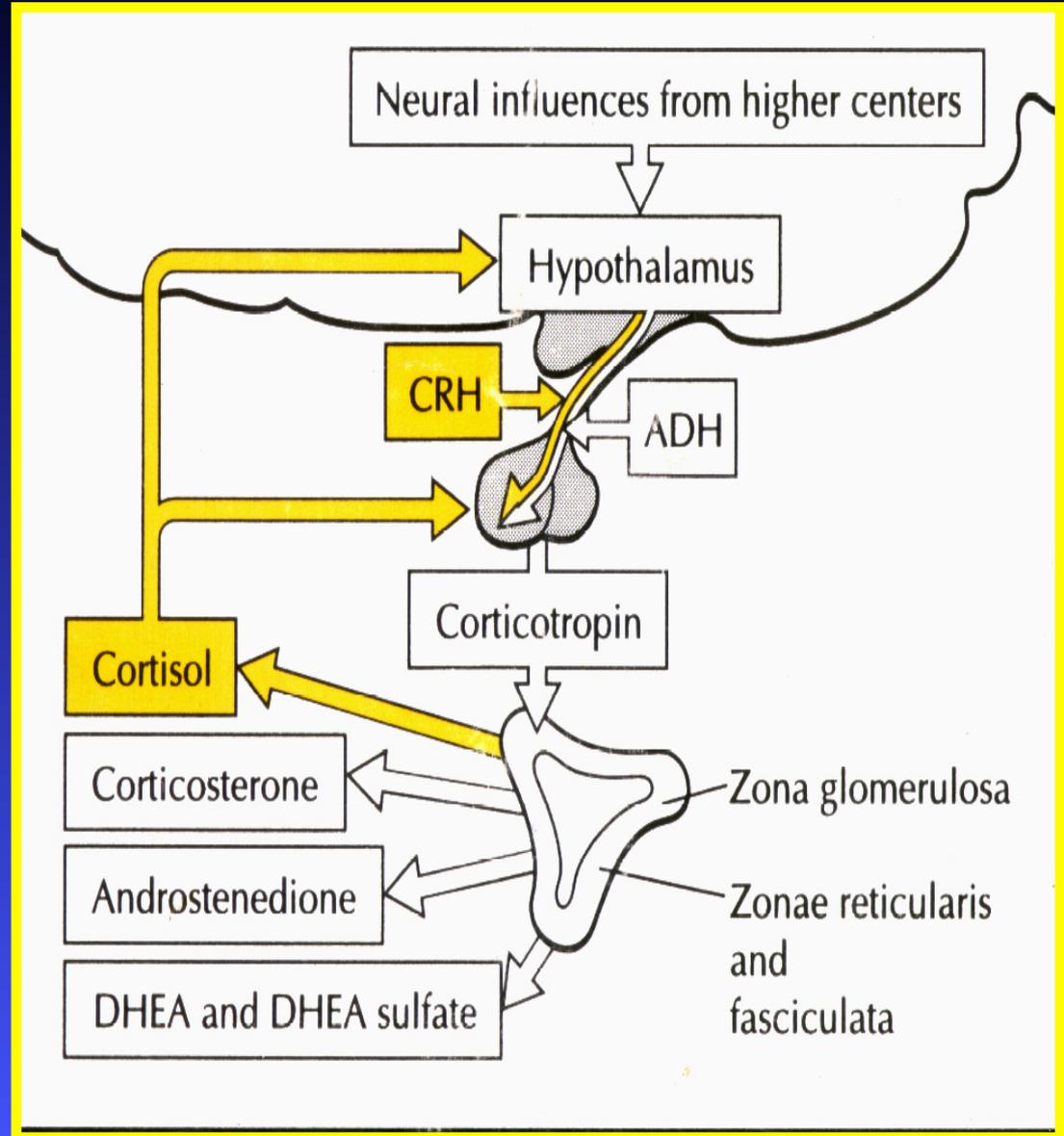




ASSE IPOTALAMO-IPOFISI-SURRENE



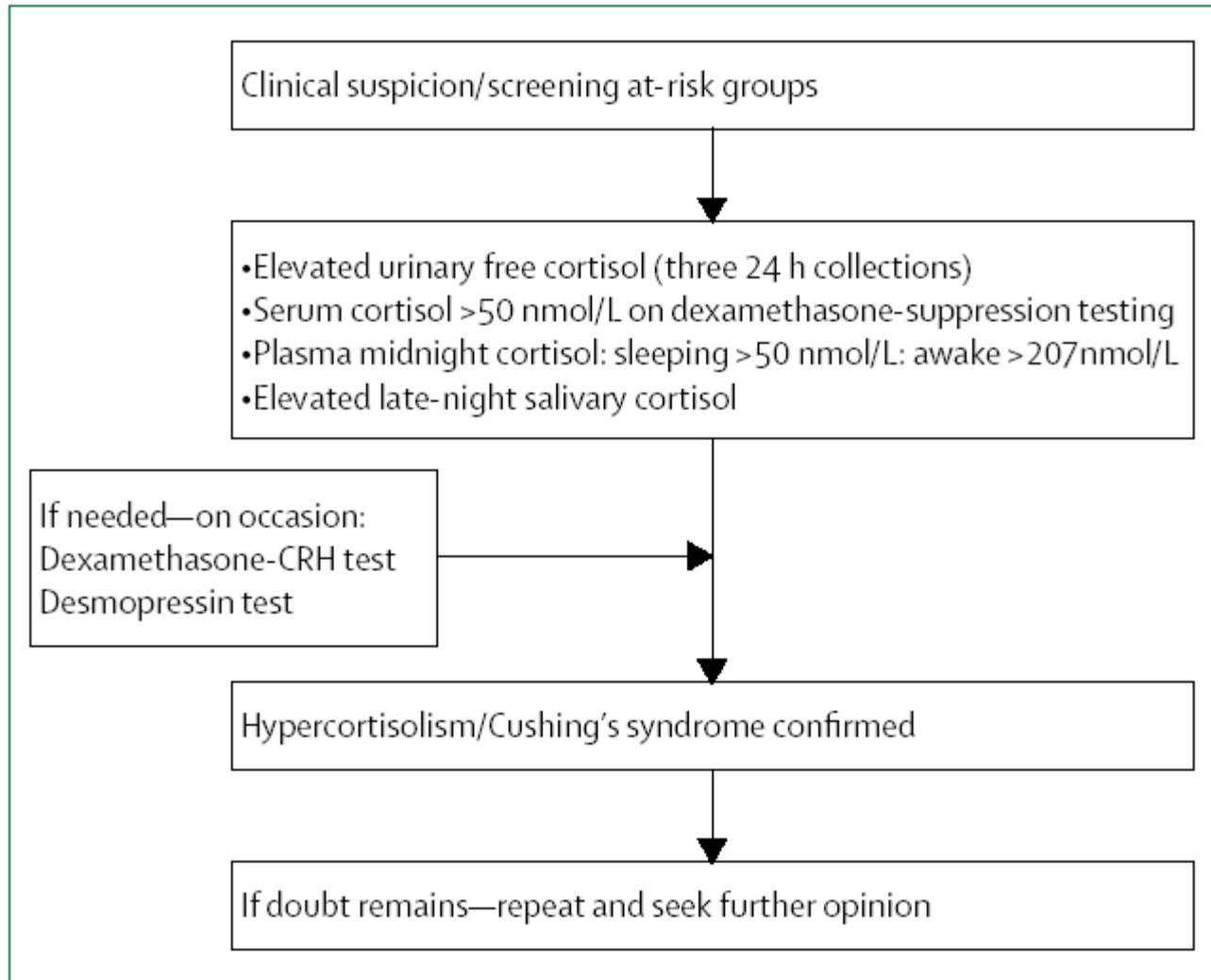
Cortisolo serico
Cortisolo urinario
Cortisolo salivare
Cortisolo capello



Cushing's syndrome

John Newell-Price, Xavier Bertagna, Ashley B Grossman, Lynnette K Nieman

Lancet 2006; 367: 1605-17



Adrenal Insufficiency



Suspected hypothalamic-pituitary disorder in ambulatory patient with no acute illness

Basal cortisol*

< 5 $\mu\text{g/dl}$
(138 nmol/l)
Probability HPAI > 92%
(95% CI 75-99)

5–13 $\mu\text{g/dl}$
(138–365 nmol/l)
Probability HPAI 40%
(95% CI 31-47)

> 13 $\mu\text{g/dl}$
(365 nmol/l)
Probability HPAI < 9%
(95% CI 3-18)

Low-dose corticotropin stimulation test (30-minute cortisol)

< 16 $\mu\text{g/dl}$
(440 nmol/l)
Probability HPAI > 83%
(95% CI 67-94)

16–22 $\mu\text{g/dl}$
(440–600 nmol/l)
Probability HPAI 33%
(95% CI 21-48)

> 22 $\mu\text{g/dl}$
(600 nmol/l)
Probability HPAI < 5%
(95% CI 1-18)

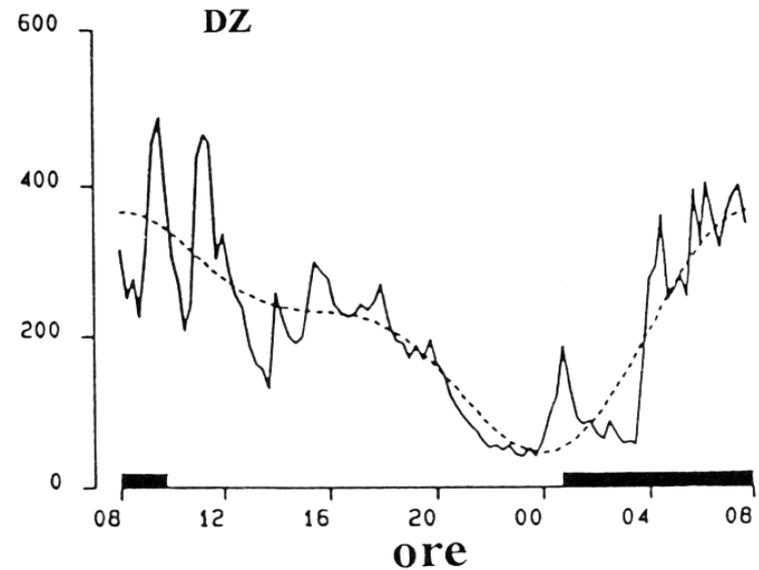
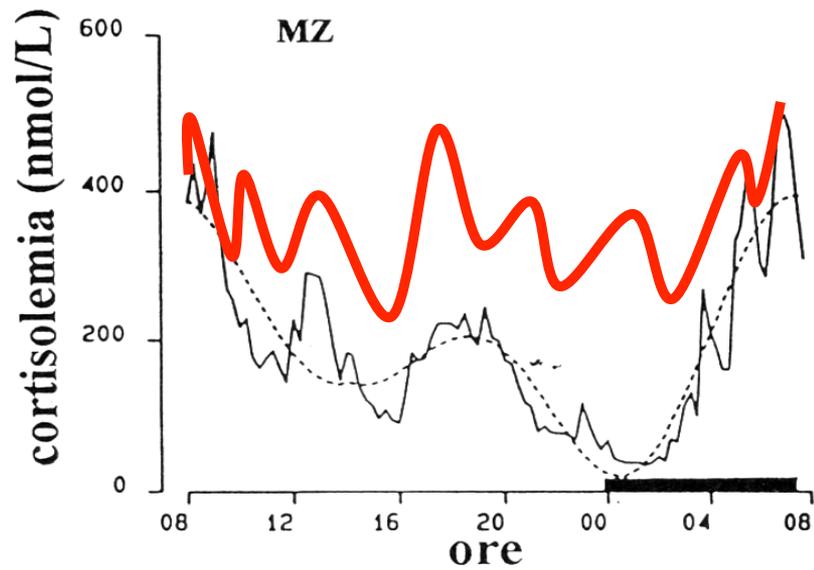
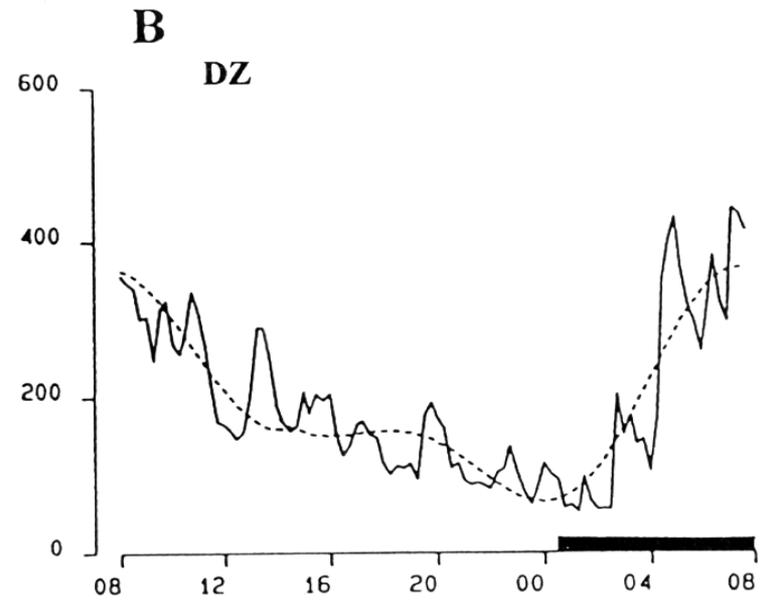
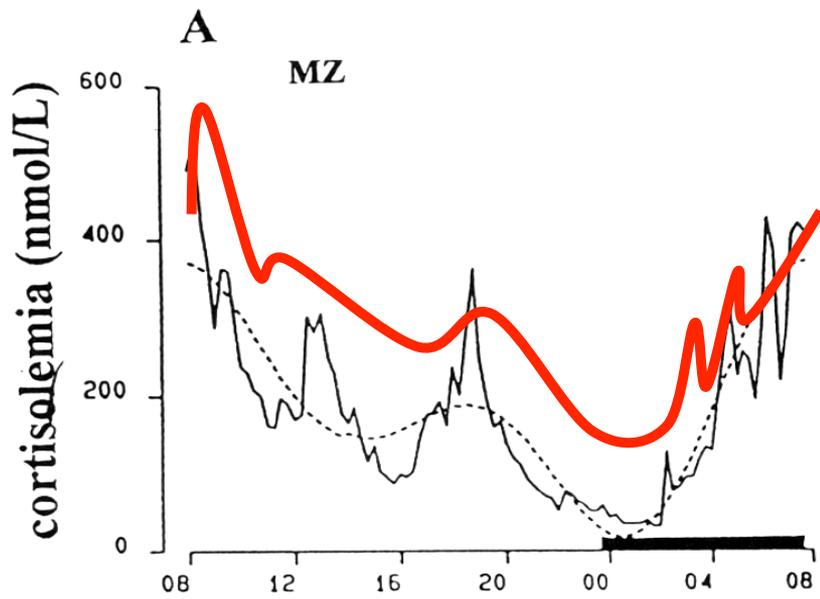
Insulin tolerance test or overnight metyrapone test

Abnormal

Normal

Glucocorticoid stress supplementation
and replacement

No glucocorticoid supplementation,
unless high clinical suspicion†

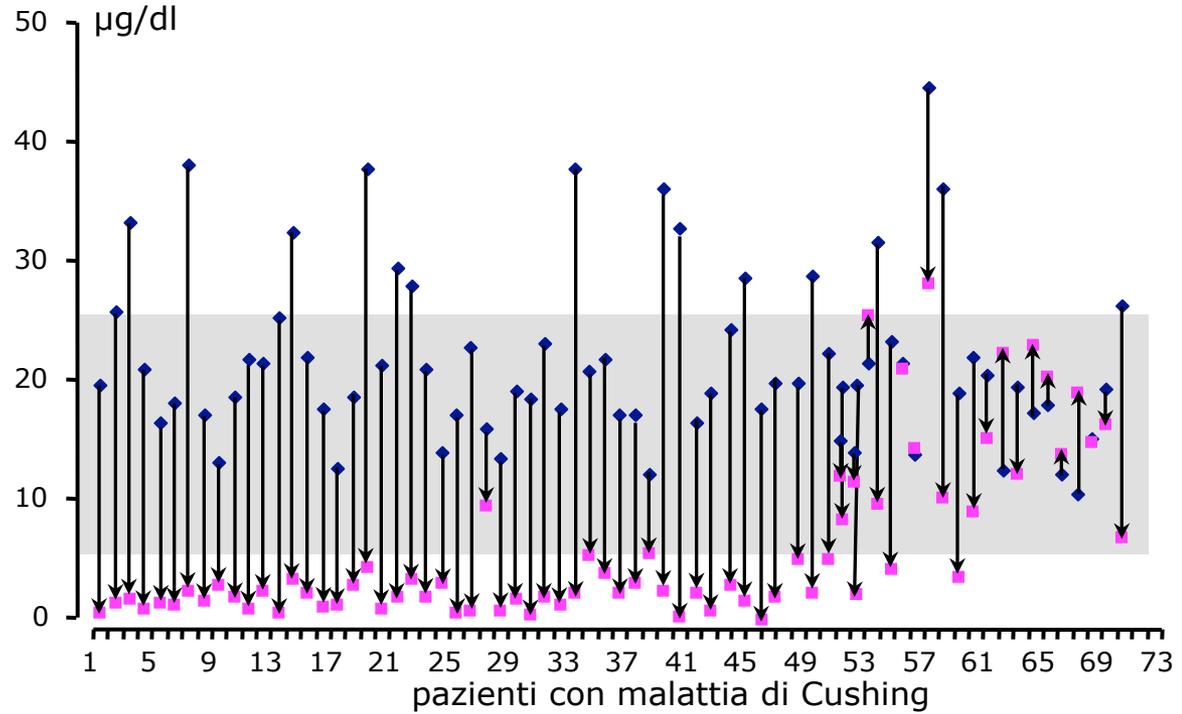
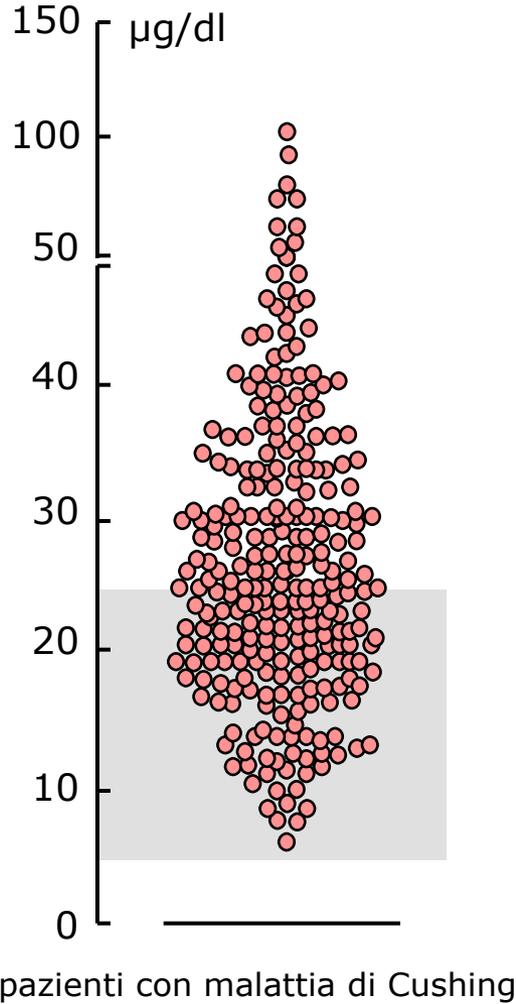


CORTISOLO SIERICO h8 - diagnosi



Diagnosi

Remissione

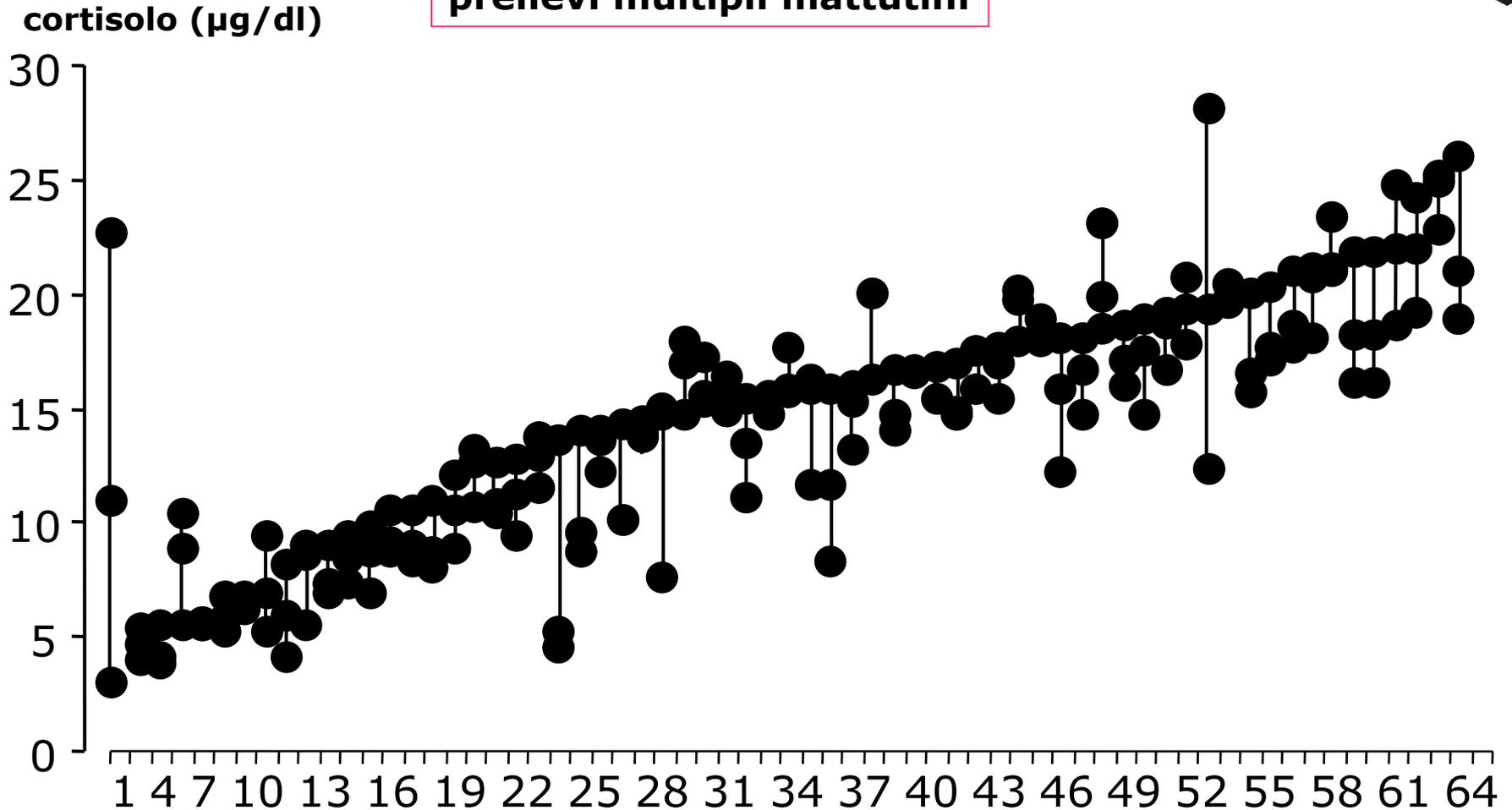


Courtesy of Pecori-Giraldi

CORTISOLO SIERICO h8 - variabilità



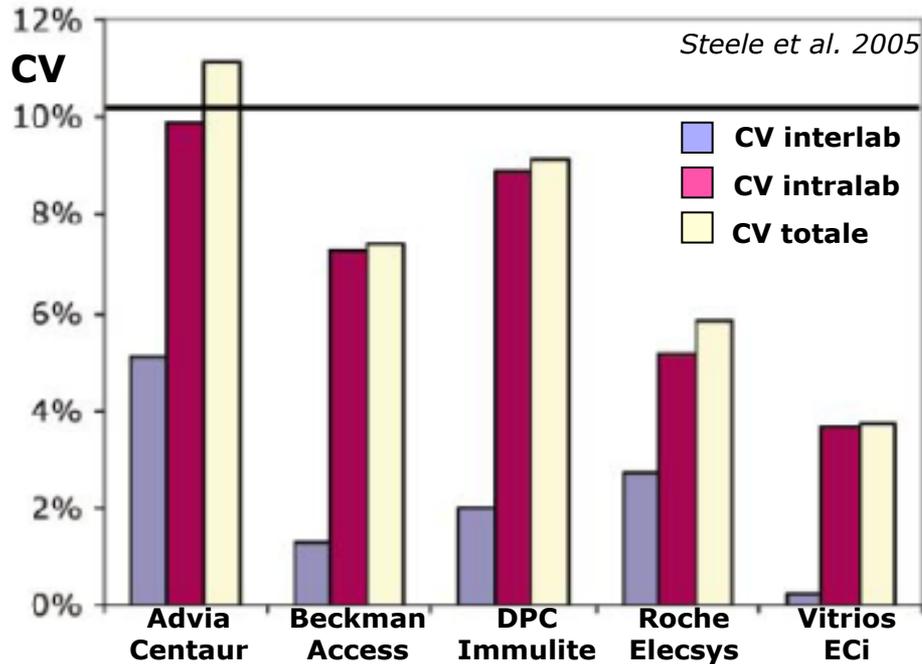
prelievi multipli mattutini



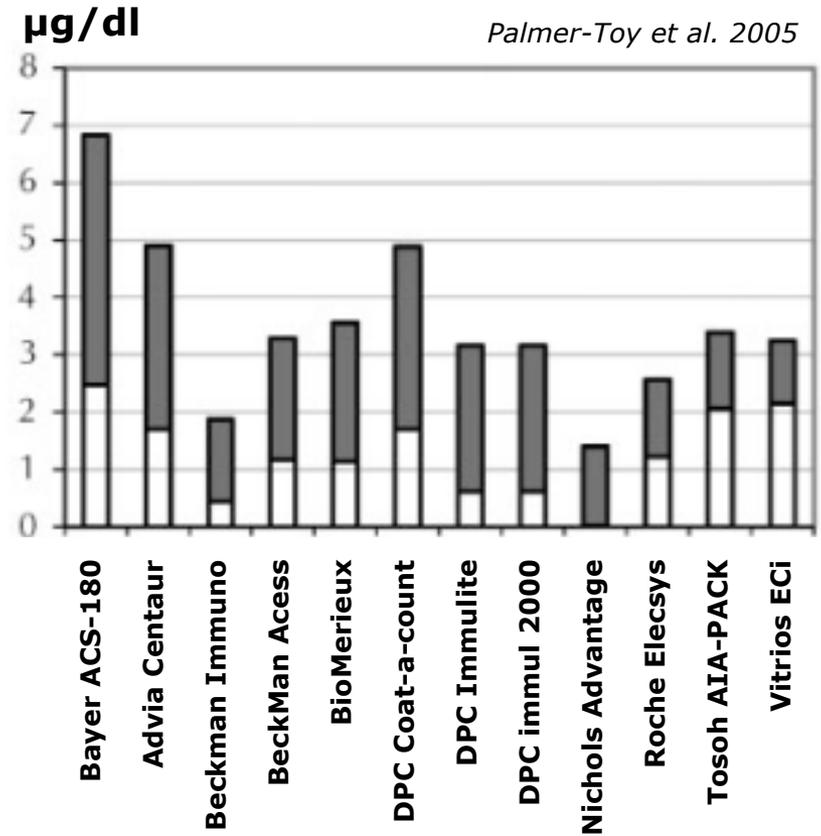
Variabilità individuale **2-13%** tra prelievi

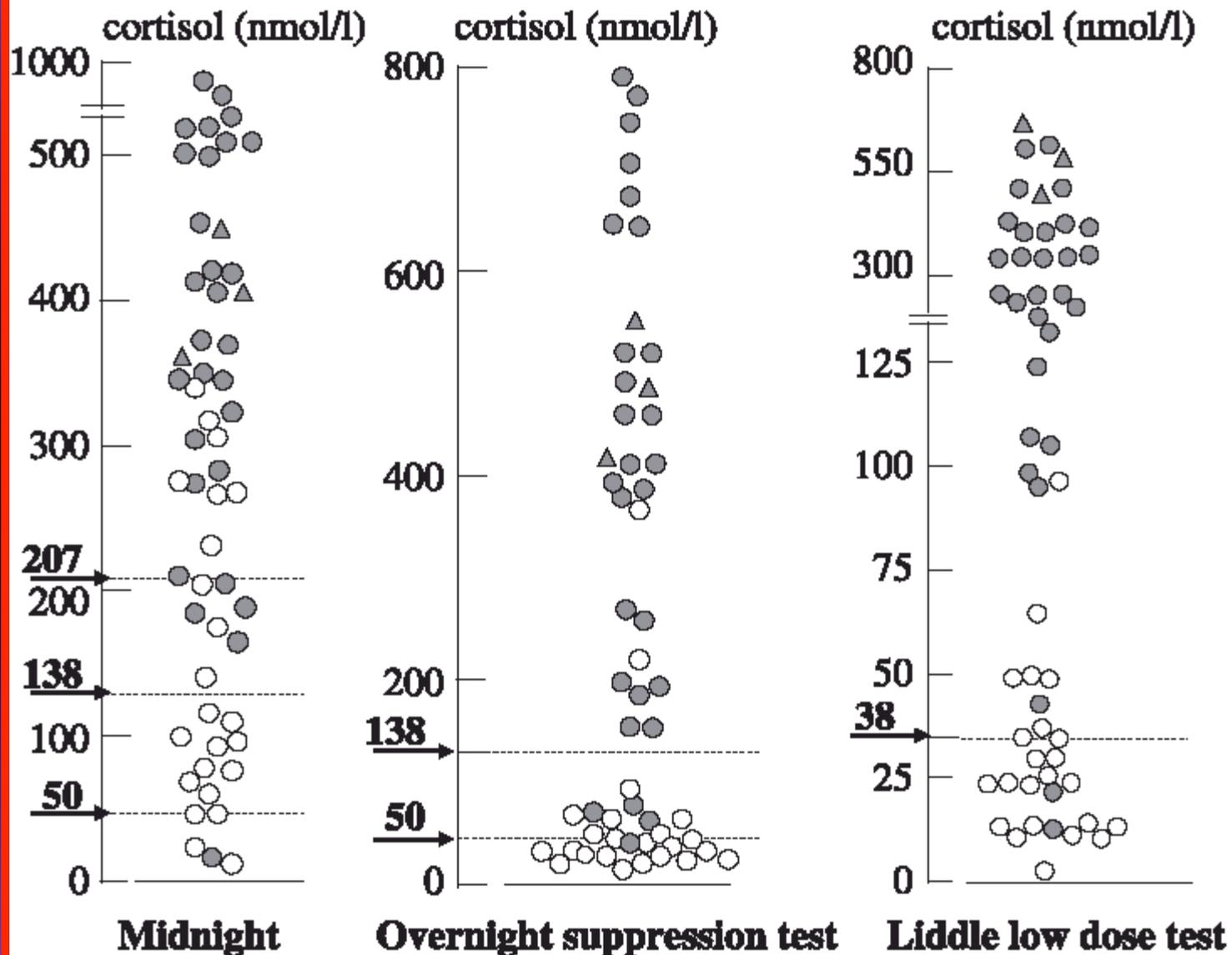
CORTISOLO SIERICO h8 - variabilità

variabilità su 330 laboratori a 6 mesi, CAP

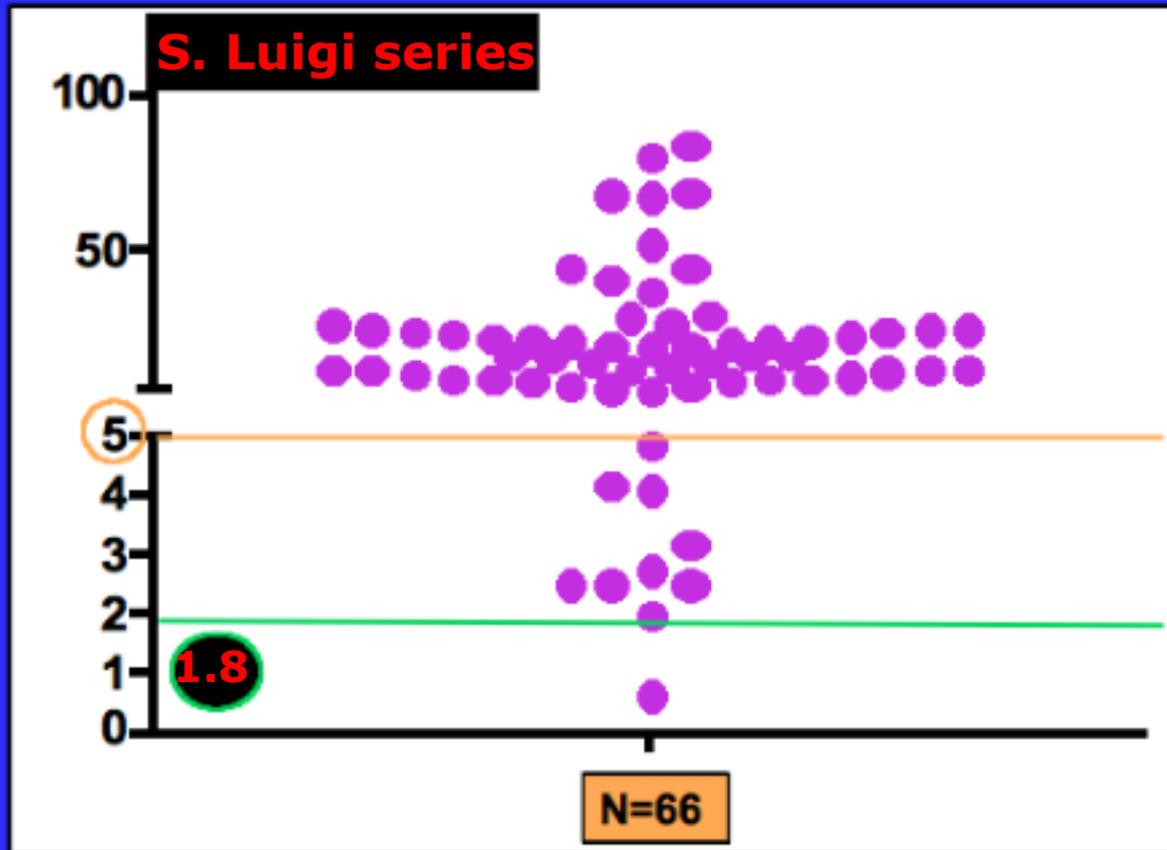


bias tra laboratori su 1015 laboratori, CAP

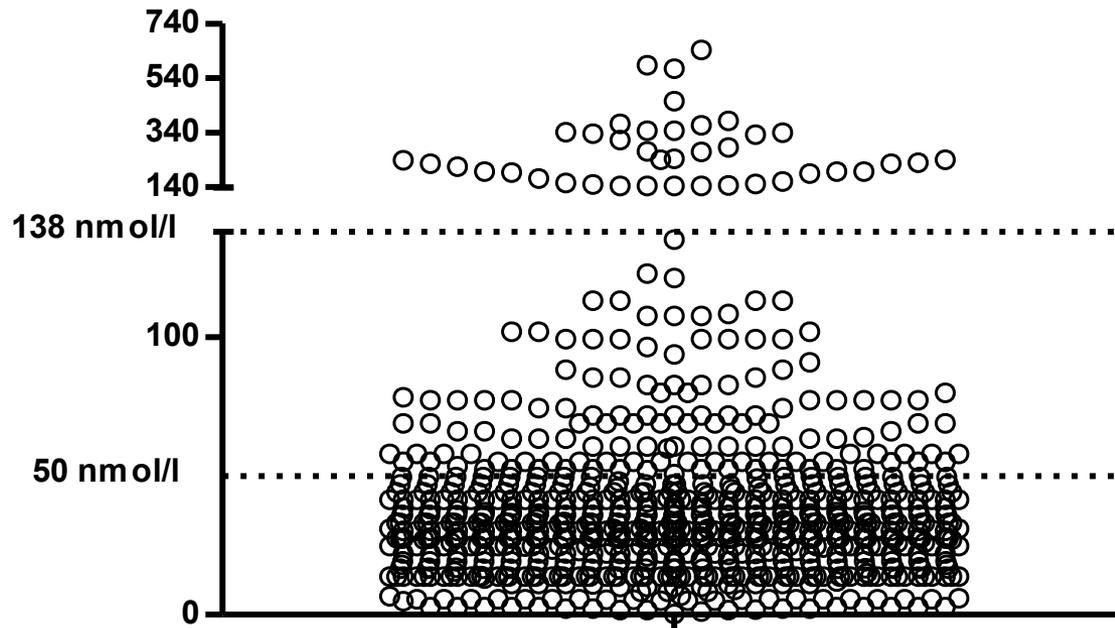




1 mg D.S.T. in Cushing's syndrome



Cortisol values after 1-mg DST.



Possibili farmaci interferenti

TABLE 3. Selected drugs that may interfere with the evaluation of tests for the diagnosis of Cushing's syndrome^a

Drugs that accelerate dexamethasone metabolism by induction of CYP 3A4

- Phenobarbital
- Phenytoin
- Carbamazepine
- Primidone
- Rifampin
- Rifapentine
- Ethosuximide
- Pioglitazone



Falsi positivi

Drugs that impair dexamethasone metabolism by inhibition of CYP 3A4

- Aprepitant/fosaprepitant
- Itraconazole
- Rilonavir
- Fluoxetine
- Diltiazem
- Cimetidine



Falsi negativi

Drugs that increase CBG and may falsely elevate cortisol results

- Estrogens
- Mitotane



Falsi positivi

Drugs that increase UFC results

- Carbamazepine (increase)
- Fenofibrate (increase if measured by HPLC)
- Some synthetic glucocorticoids (immunoassays)
- Drugs that inhibit 11 β -HSD2 (licorice, carbenoxolone)



Falsi positivi

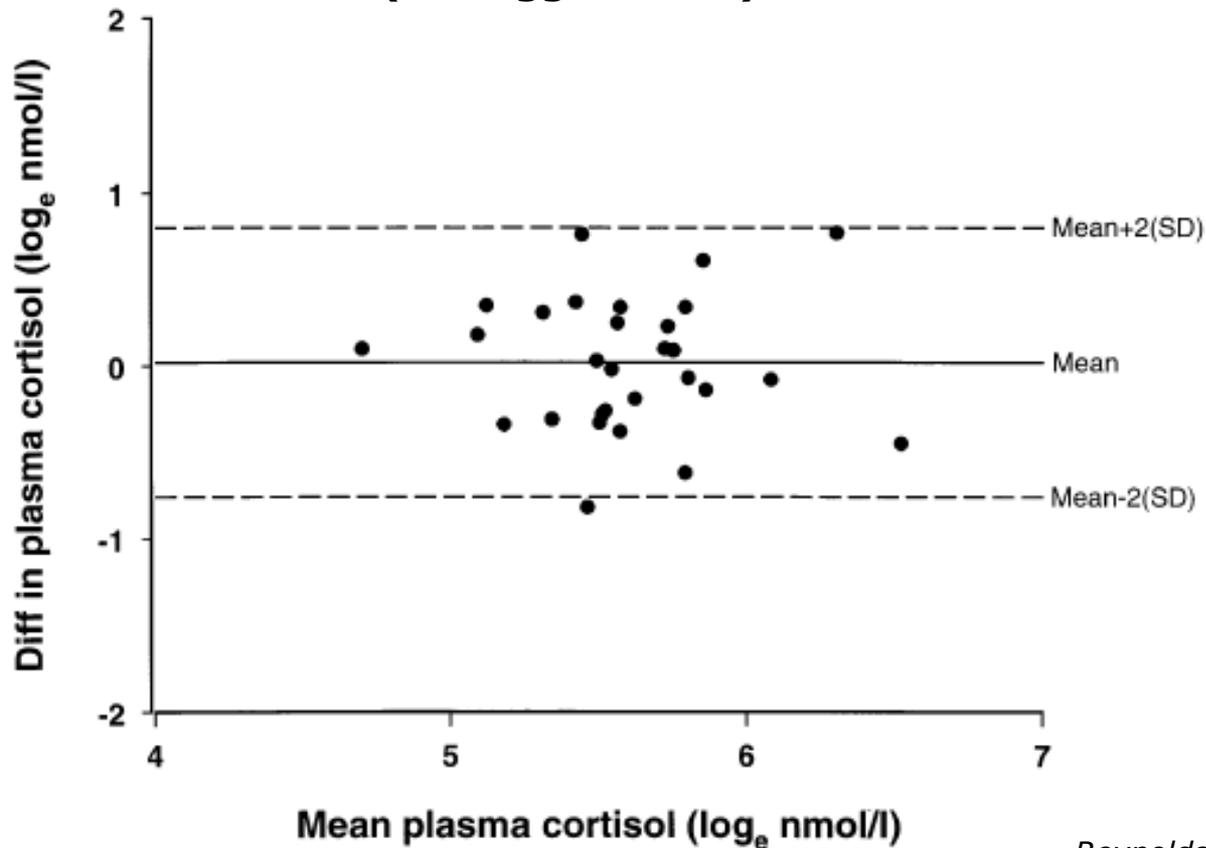
^aThis should not be considered a complete list of potential drug interactions. Data regarding CYP3A4 obtained from <http://medicine.tupst.edu/rockstar/table.htm>.

CORTISOLO dopo OST - variabilità



OST ripetuto dopo 1-10 settimane

(29 soggetti sani)



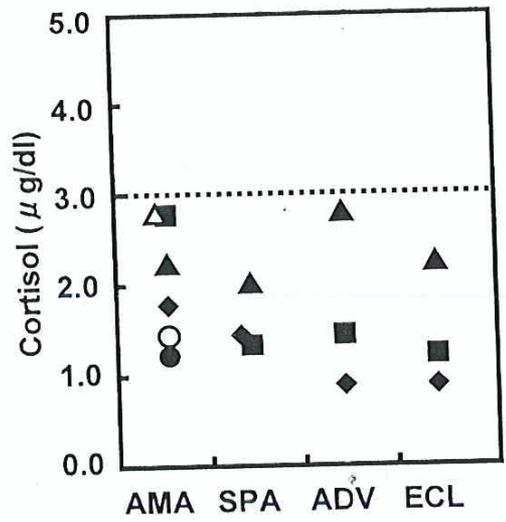
Reynolds et al 1998

variabilità: 50% a 200% del primo risultato

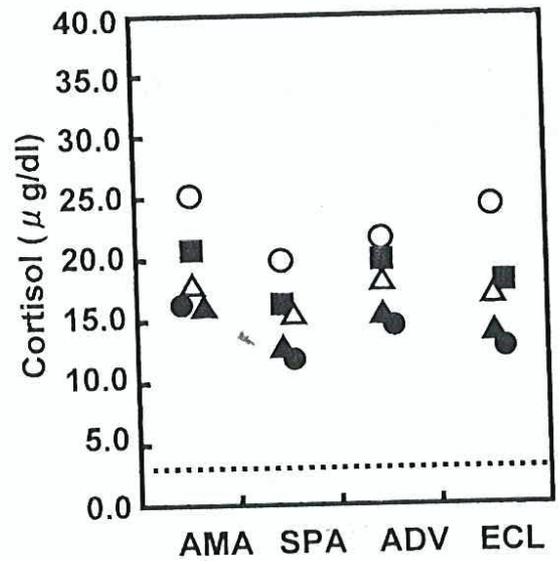
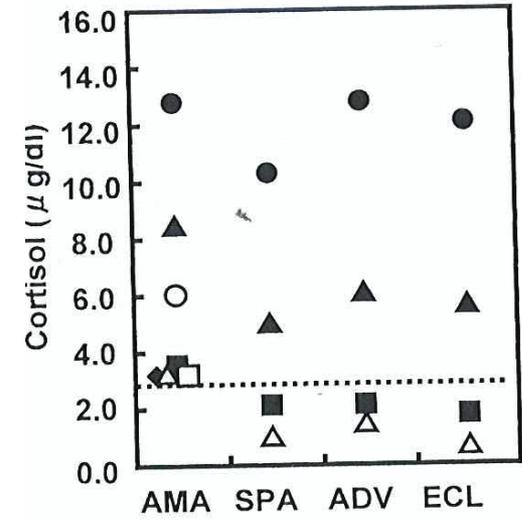
CORTISOLO dopo OST - variabilità



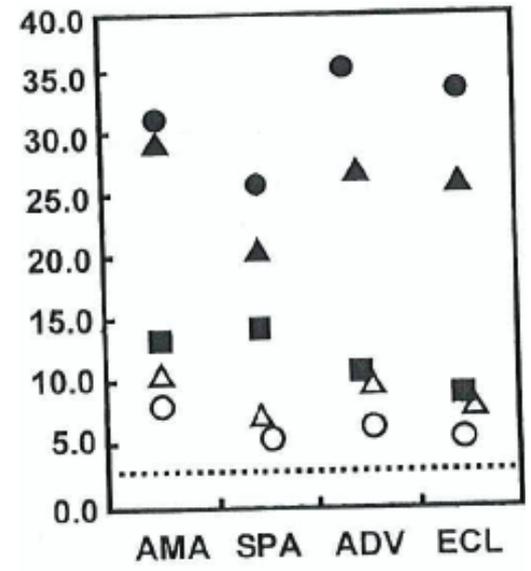
confronto cortisolo dopo OST con 4 kit



Cushing preclinico (n=7) →
← controlli (n=6)



malattia di Cushing (n=5) →
← adenoma surrenalico (n=5)



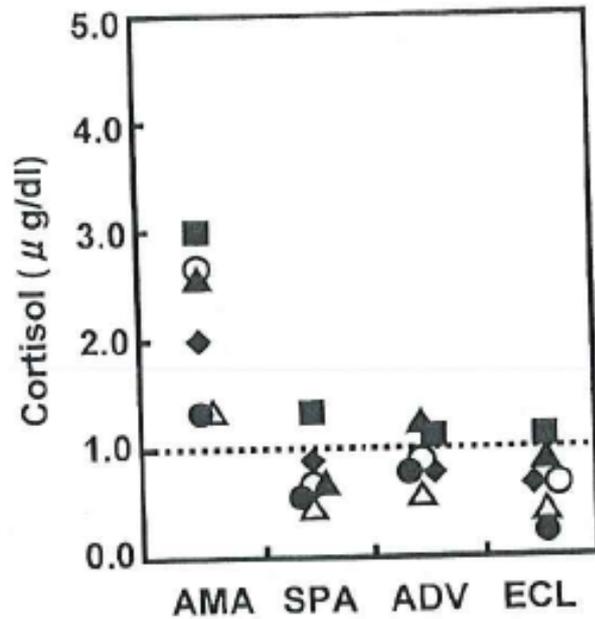
Odagiri et al 2004

CORTISOLO dopo 8 mg DEX - variabilità

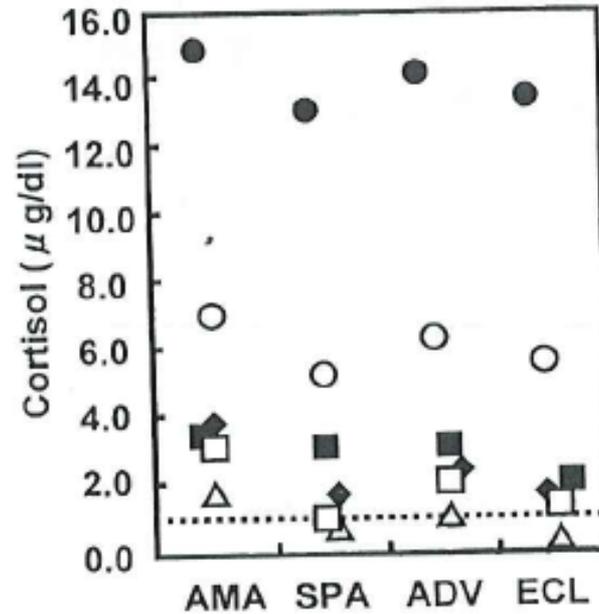


confronto cortisolo dopo 8 mg DEX con 4 kit

controlli (n=6)



Cushing preclinico (n=7)

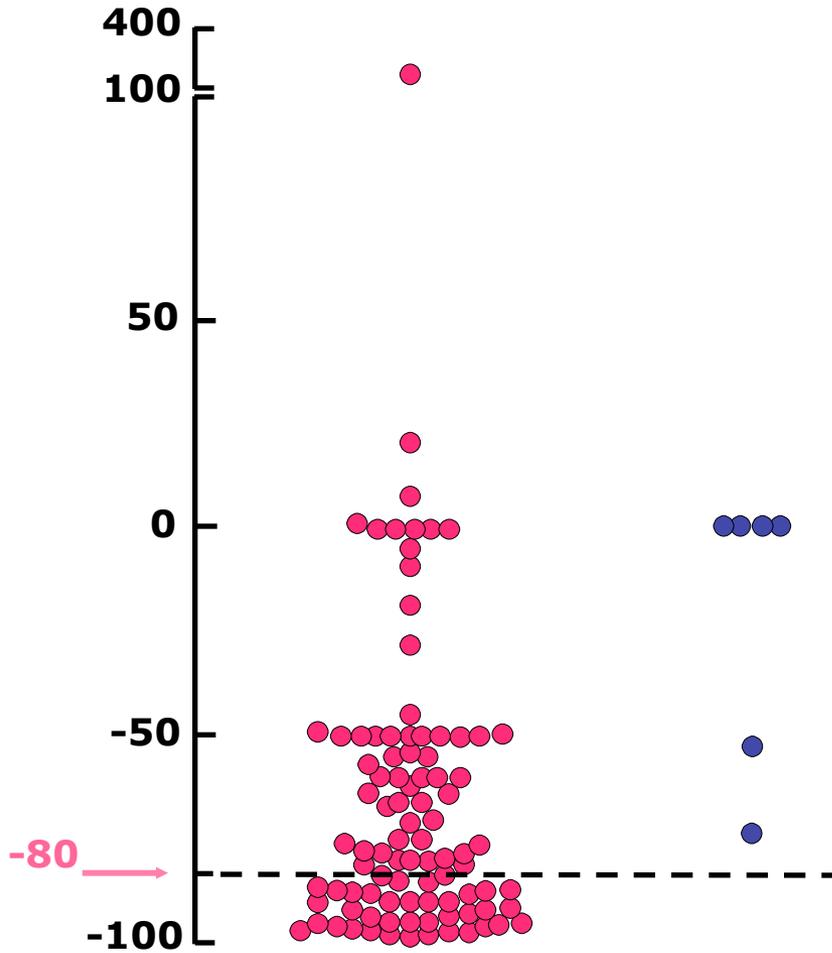


CORTISOLO dopo 8 mg DEX

Diagnosi Differenziale



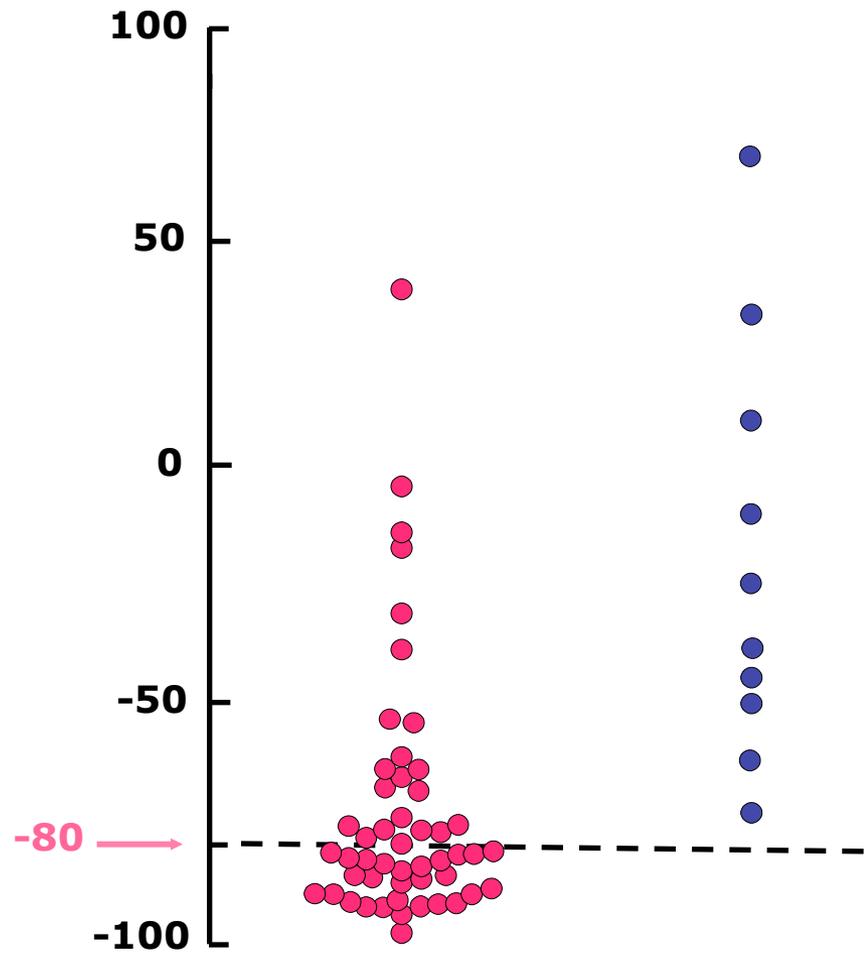
Cortisolo libero urinario (% basale)



Malattia di Cushing

ACTH ectopico

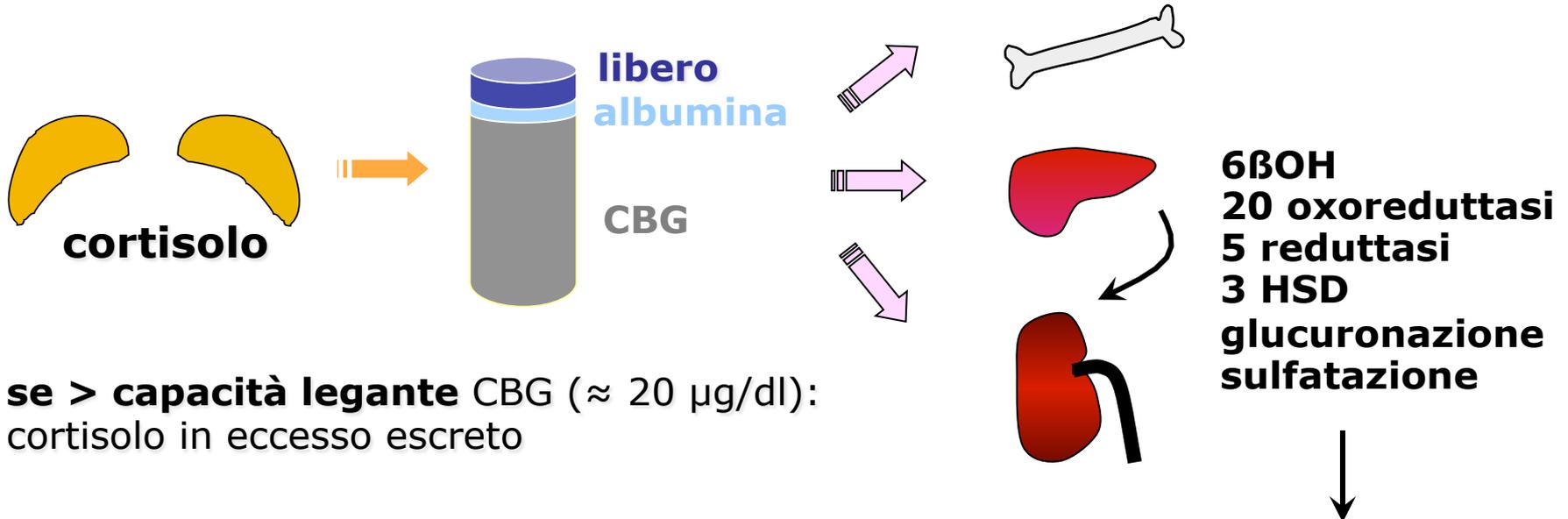
Cortisolo plasmatico (% basale)



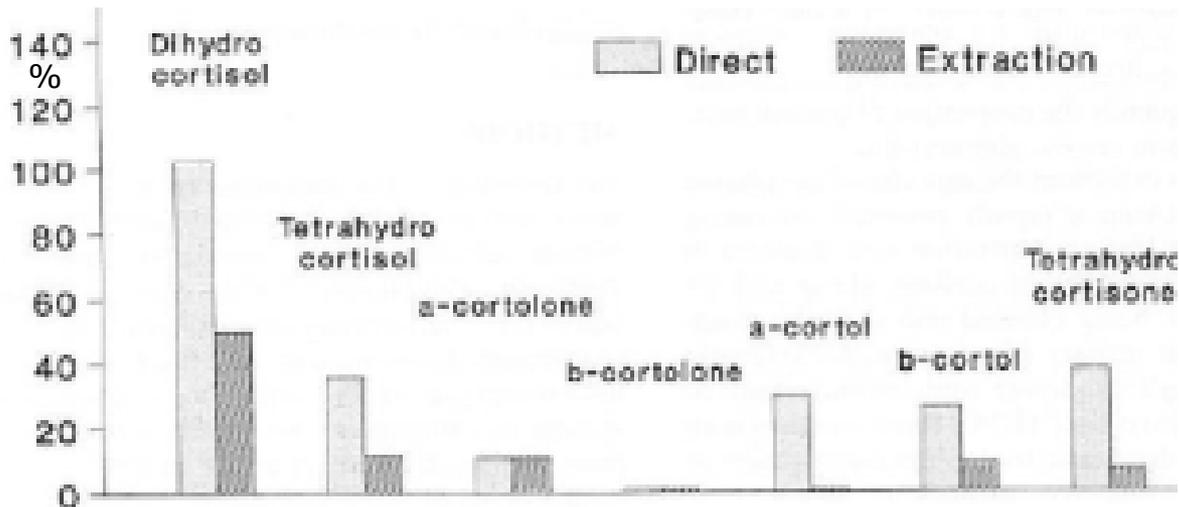
Malattia di Cushing

ACTH ectopico

CORTISOLO LIBERO URINARIO

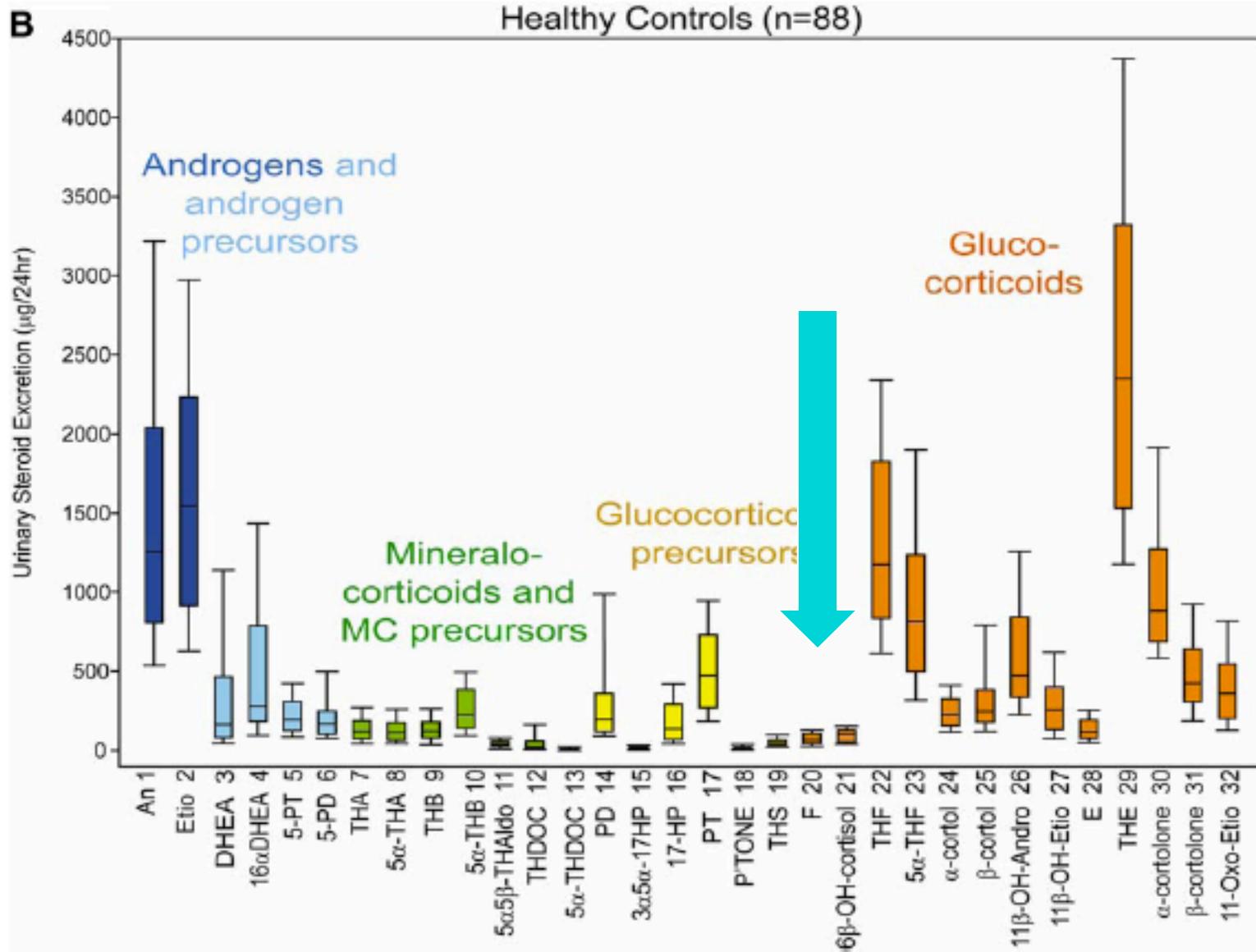


UFC **metaboliti**



5 tetraidrocortisolo
20 diidro cortisolo
6β idrocortisolo
glucuronati
etiocolanone
cortoli/acido cortolico

Migliardi et al, 2010



METODICHE di DOSAGGIO CORTISOLO LIBERO URINARIO



IMMUNOCHEMICHE:

RIA, EIA, ECLIA

- diretti
- estrattivi (dopo diclorometano)

Metodica uguale cortisolo sierico

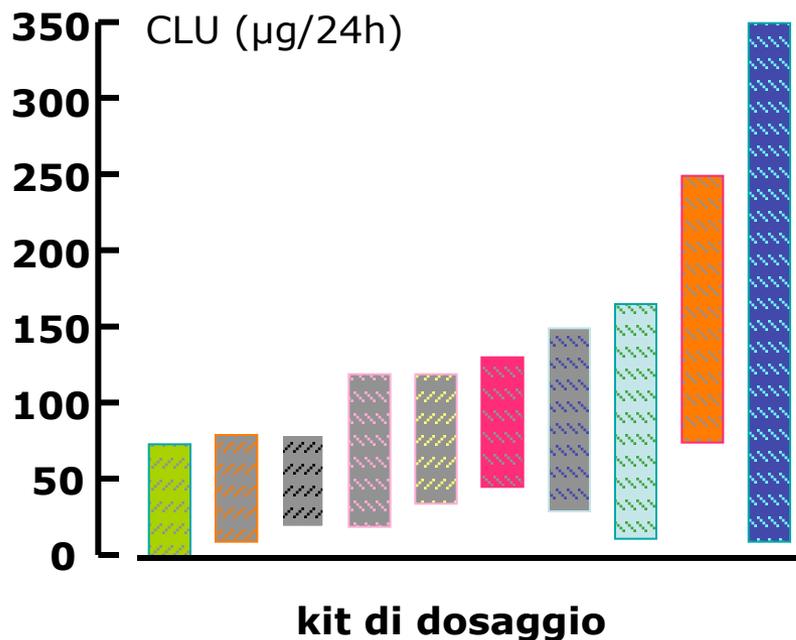
MA concentrazione di CLU è molto più bassa di quella di altre molecole steroidee interferenti

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**

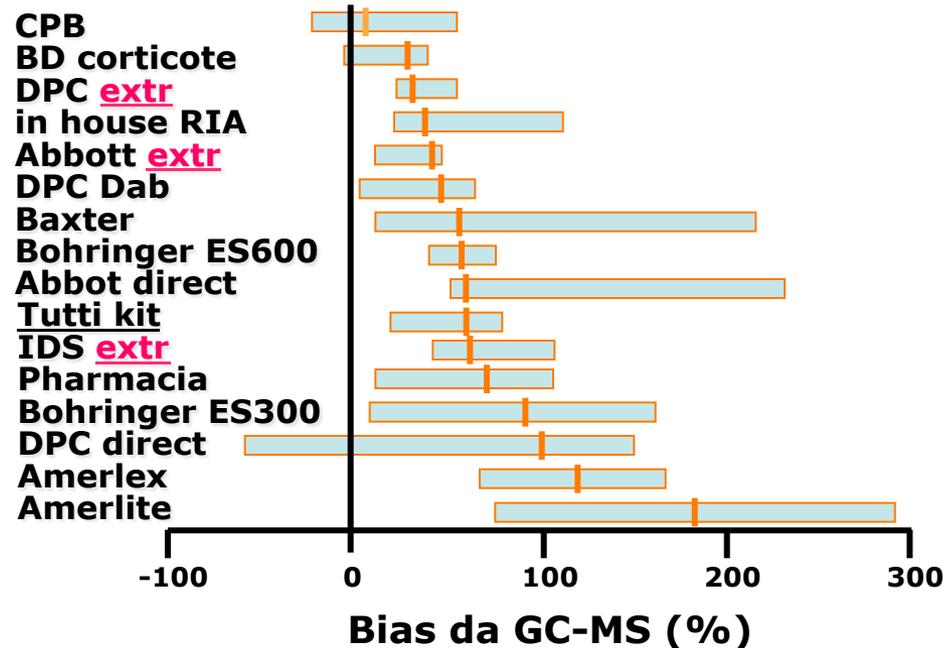
DOSAGGI del CORTISOLE LIBERO URINARIO

RANGE DI NORMA



Gruppo di studio HPA

ACCURATEZZA



UKNEQAS - 1998



METODICHE di DOSAGGIO

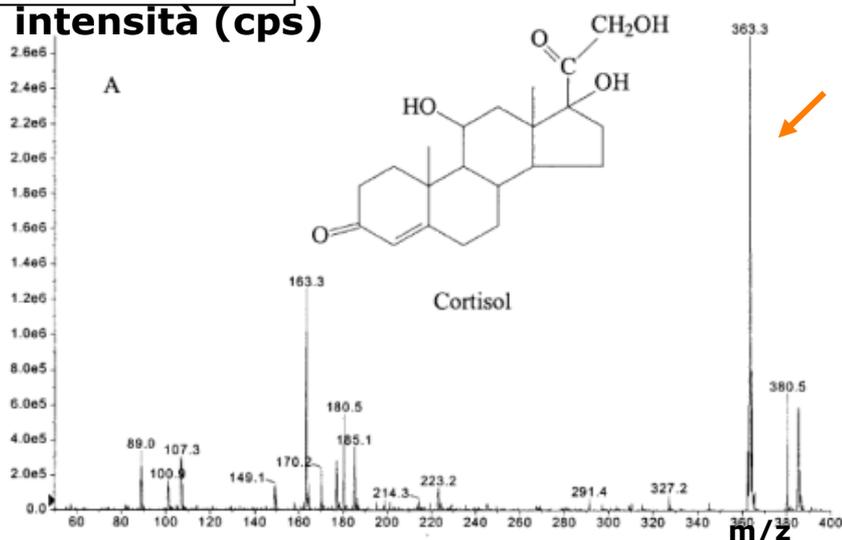


CROMATOGRAFICHE:

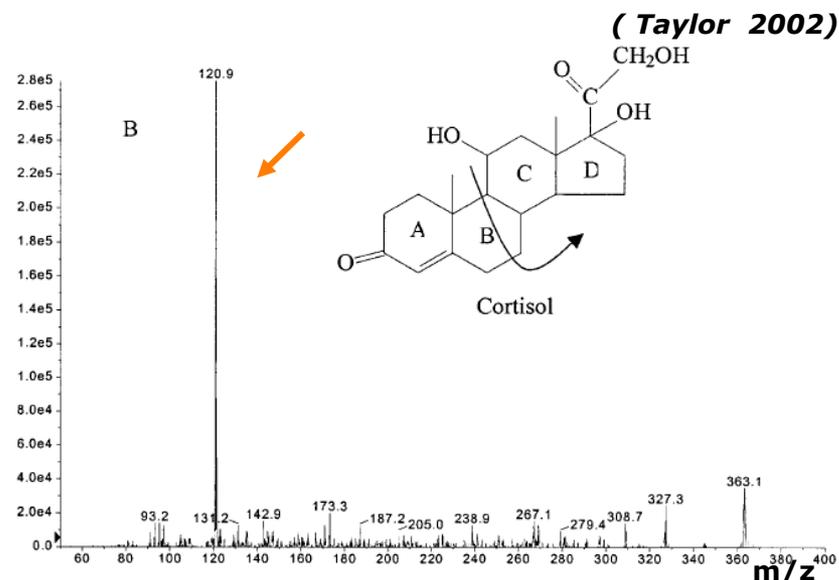
- cromatografia liquida con rilevazione ultravioletti (LC-UV)
- cromatografia liquida ad elevata prestazione e rilevazione ultravioletti (HPLC-UV)
- cromatografia liquida e spettrometria di massa (LC-MS)
- cromatografia liquida e spettrometria di massa tandem (LC-MS/MS)
- cromatografia gassosa e spettrometria di massa (GC-MS)

LC-MS/MS

intensità (cps)



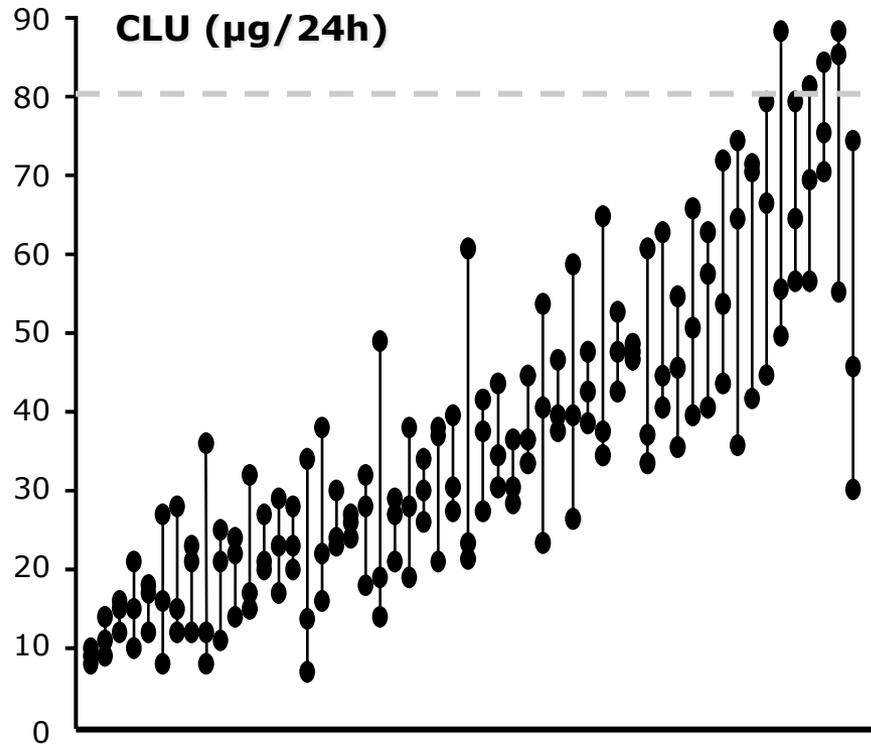
prima ionizzazione elettrospray



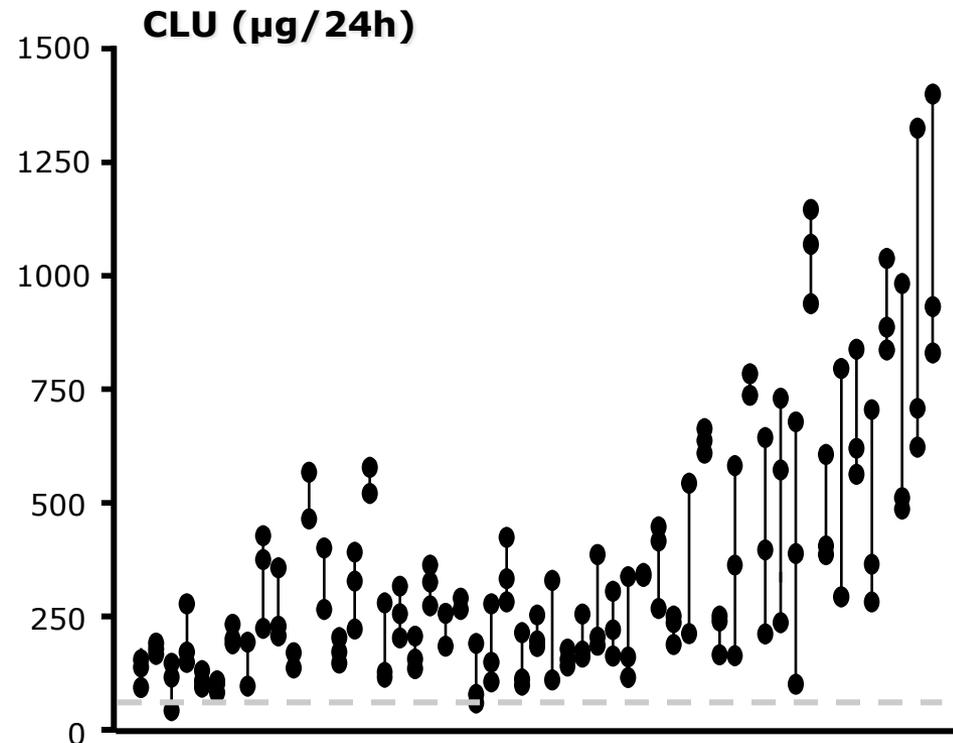
dopo ionizzazione elettrospray

VARIABILITA' dell'ESCREZIONE di CORTISOLO LIBERO URINARIO

soggetti normali



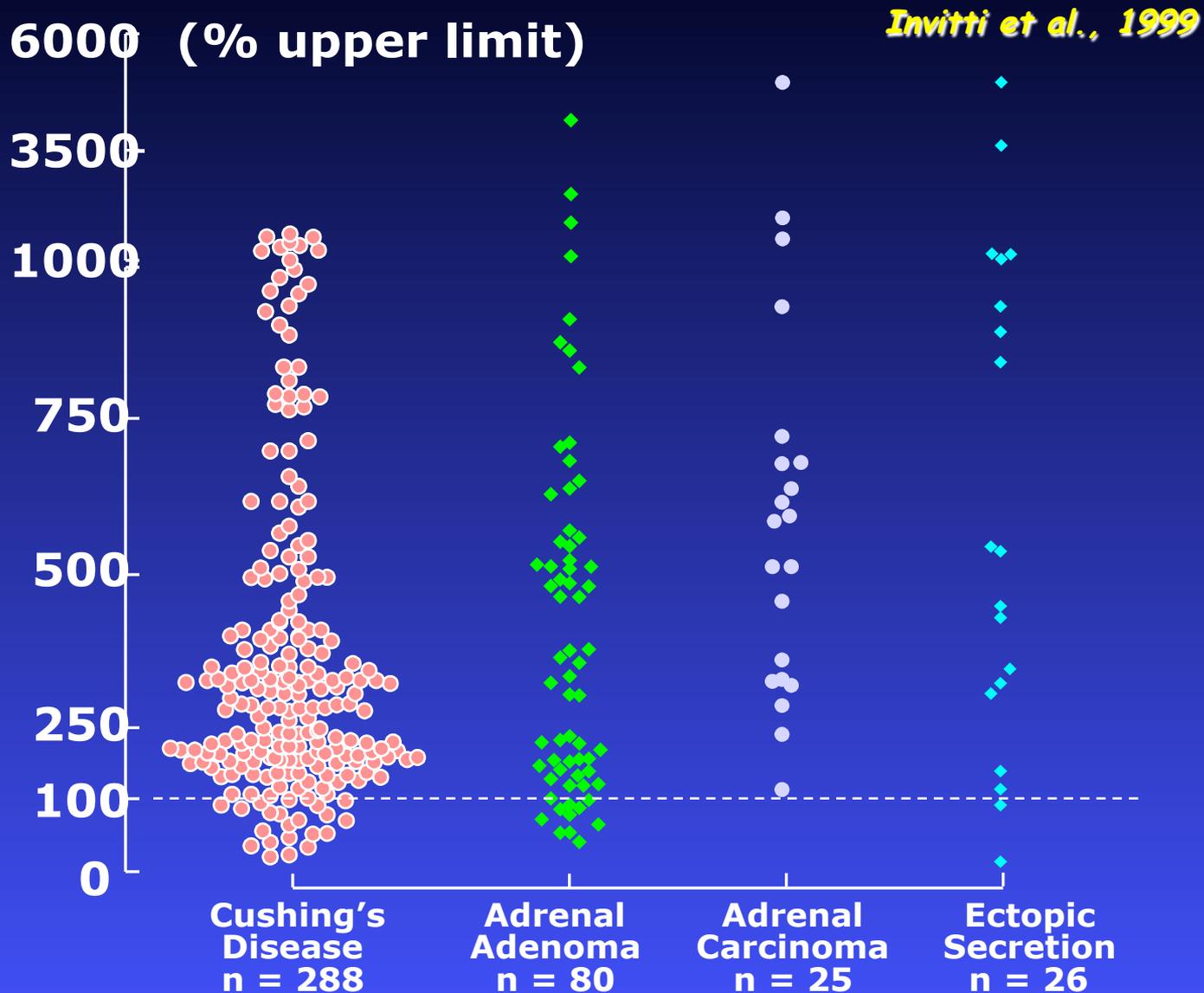
pazienti con sindrome di Cushing



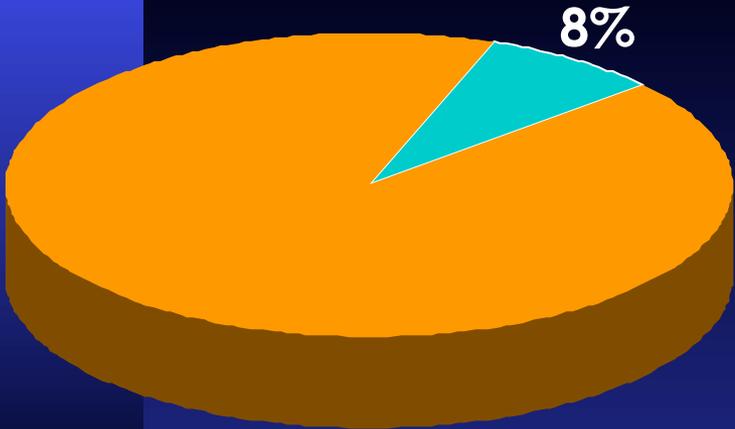
Variabilità individuale **41-46%** da giorno a giorno (*Deutschbein 2011*)



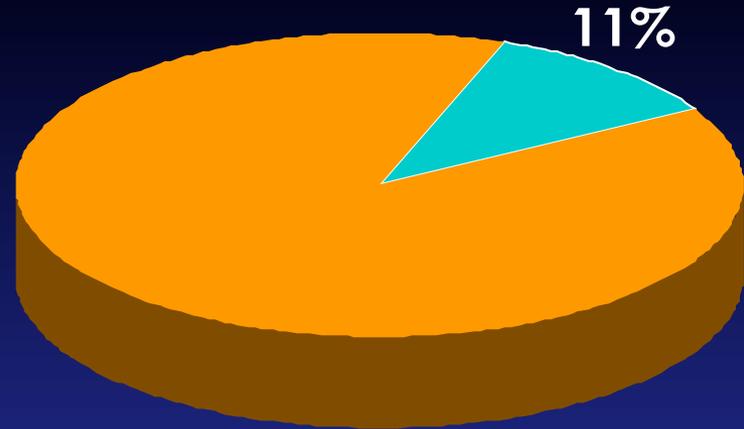
URINARY FREE CORTISOL IN PATIENTS WITH CUSHING'S SYNDROME



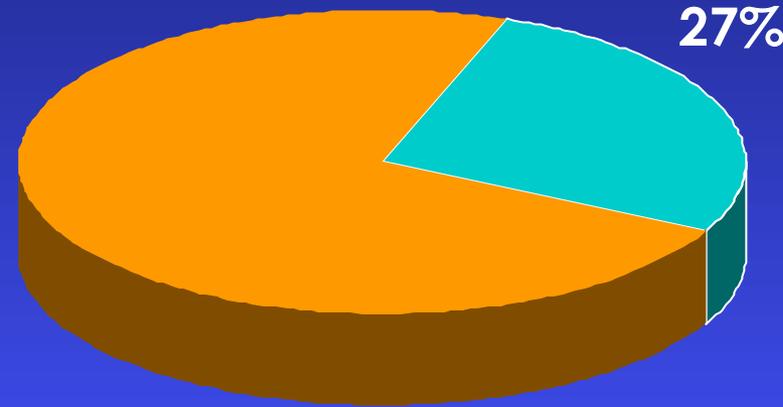
Cortisolo a mezzanotte (8.3 $\mu\text{g/dL}$)



DST 1 mg (4 $\mu\text{g/dL}$)



CLU (238 $\mu\text{g}/24$ ore)





100% SENSITIVITY or 100% SPECIFICITY

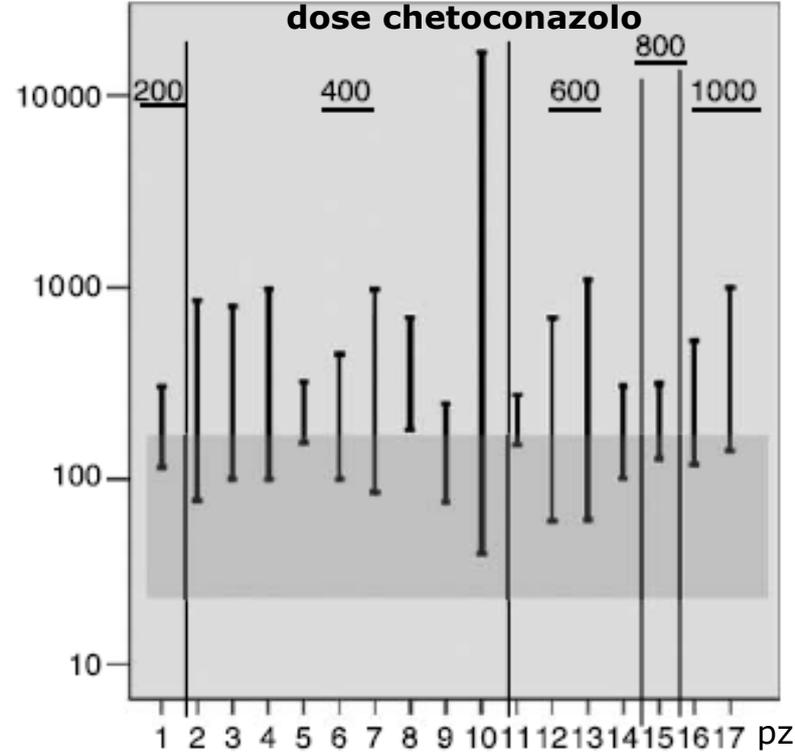
	100% sensitivity [specificity]	100% specificity [sensitivity]
Midnight cortisol ($\mu\text{g}/\text{dL}$)	3.7 [67.9 (47.7- 84.1)]	11.0 [84.9 (74.6- 92.2)]
DST ($\mu\text{g}/\text{dL}$)	1.5 [68.2 (45.1- 86.1)]	14.4 [49.2 (36.6- 61.9)]
UFC ($\mu\text{g}/24\text{ h}$)	38 [18.5 (6.4- 38.1)]	277 [63.4 (51.1- 74.5)]

CORTISOLO LIBERO URINARIO dopo terapia



UFC (nmol/24h)

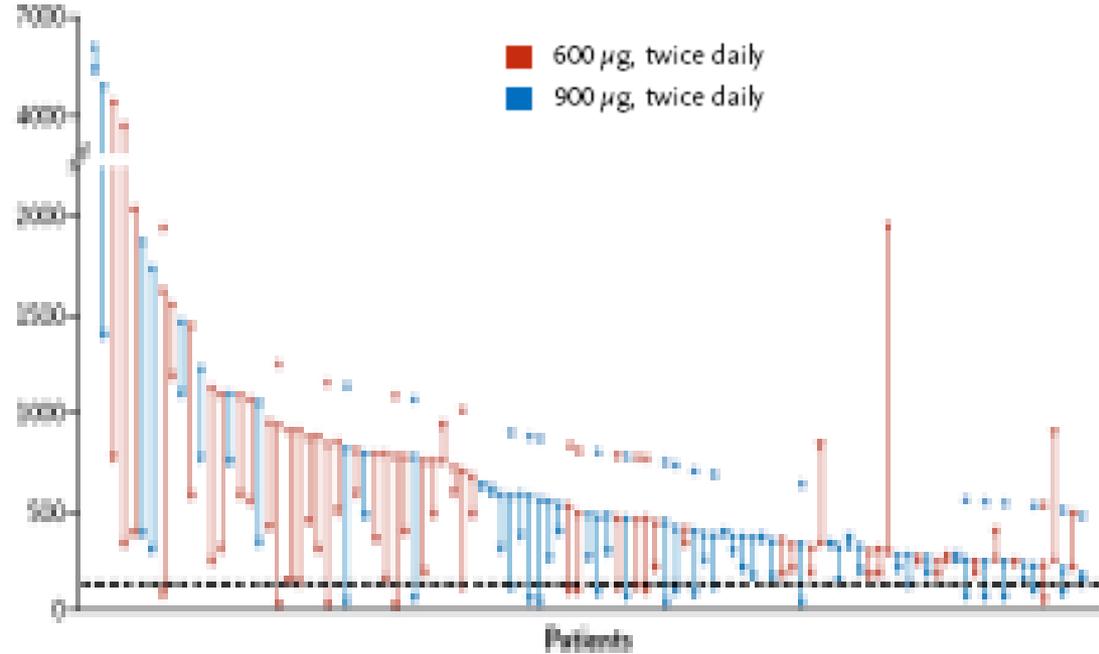
dose chetoconazolo



Castinetti et al 2008

efficace ≈ 50-90% pz

UFC (nmol/24h)



Colao et al 2012

efficace ≈ 25% pz

