Il caso dell'aldosterone:
fra certezze e pitfalls

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12° congresso Nazionale AME

6th Joint Meeting with AACE

Punti chiave

1. Impiego diagnostico del dosaggio dell'aldosterone e della renina

2. Il rapporto aldosterone/renina (ARR)

3. Criticità del dosaggio dell'aldosterone e della renina
   Preanalitiche
   Analitiche
   Postanalitiche

Il rapporto A/R può essere utilizzato
per la rapida classificazione di tutti i disordini
del sistema renina-aldosterone

Iperaldosteronismo primario (PA) e secondario
Ipoaldosteronismo primario e secondario

The Simultaneous Measurement of Plasma-Aldosterone- and -Renin-Concentration Allows Rapid Classification of all Disorders of the Renin-Aldosterone System

Author   S. Diederich[1,2], K. Mai[1], V. Bähr[1], S. Helffrich[2], A. Pfeiffer[1], F. H. Perschel[3]

Impiego diagnostico dell'ARR

Clinical Practice Guideline

Case Detection, Diagnosis, and Treatment of Patients with Primary Aldosteronism: An Endocrine Society Clinical Practice Guideline

John W. Funder, Robert M. Carey, Carlos Fardella, Celso E. Gomez-Sanchez, Franco Mantero, Michael Stowasser, William F. Young Jr., and Victor M. Montori*

Patients with relatively high prevalence of PA

Stage 2 (>160-179 mmHg/100-109 mmHg) or stage 3 (>180/110 mmHg) hypertension
Drug resistant hypertension
Hypertension and spontaneous or diuretic-induced hypokalemia
Hypertension with adrenal incidentaloma
Hypertension and family history of early-onset hypertension or cerebravascular accident at young age (<40 years)

Hypertensive first-degree relatives of patients with PA

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The aldosterone to renin ratio (ARR) is currently the most reliable available mean of screening for PA
ARR is superior to mesurement of potassium or aldosterone (lower sensitivity) or renin (lower specificity) in isolation (Hiramatsu, 1981; McKenna 1991; Stowasser, 2010)

A Screening Test to Identify Aldosterone-Producing Adenoma by Measuring Plasma Renin Activity

Results in Hypertensive Patients

Kunihide Hiramatsu, MD; Takashi Yamada, MD; Yaichiro Yukimura, MD; Ichiro Komiya, MD;
Kazuo Ichikawa, MD; Masaki Ishihara, MD; Hajime Nagata, MD; Tomio Izumiyama, MD

Review Article

Laboratory Investigation of Primary Aldosteronism

*Michael Stowasser, Paul J Taylor, Eduardo Ementa, Ashraf H Al-Asaly Ahmed, Richard D Gordon
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ARR and PA

Clinical Practice Guideline

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ARR is not without false positive and negative
ARR should be regarded as a detection (screening) test
ARR should be repeated if the initial results are inconclusive or difficult to interpret because of suboptimal sampling conditions

ARR:
variabili preanalitiche

Test characteristics of the aldosterone-to-renin ratio as a screening test for primary aldosteronism

J Hypertens 2013

Pieter M. Jansen[a], Bert-Jan H. van den Born[b], Wijnanda J. Frenkel[b], Emile L.E. de Bruijne[c], Jaap Deinum[d], Michiel N. Kerstens[e], Yvo M. Smulders[f], Arend Jan Woittiez[g], Johanna A.M. Wijbenga[h], Robert Zietse[a], A.H. Jan Danser[a], and Anton H. van den Meiracker[a]

Factors Affecting the Aldosterone/Renin Ratio

Horm Metab Res 2012

Authors   M. Stowasser, A. H. Ahmed, E. Pimenta, P. J. Taylor, R. D. Gordon

Assay Characteristics Influence the Aldosterone to Renin Ratio as a Screening Tool for Primary Aldosteronism: Results of the German Conn's Registry

Horm Metab Res 2013

Authors   E. Fischer[1], S. Reuschl[1], M. Quinkler[2], L. C. Rump[3], S. Hahner[4], M. Bidlingmaier[1], M. Reincke[1]
For the Participants of the German Conn's Registry – Else Kröner-Fresenius-Hyperaldosteronism Registry

Postura
Ora del prelievo
Età
Sesso
Dieta sodica
Concentrazione del potassio
Farmaci

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Postura:

    1 Campione dopo 5-15 minuti in posizione seduta

    No: 2 campioni (ortostatismo/1 ora-clinostatismo)

Ora:

    Metà mattina (ritmo circadiano di ACTH)

    No: pomeriggio

Reference Intervals for Aldosterone, Renin, and the Aldosterone-to-Renin Ratio in the Population-based Study of Health in Pomerania (SHIP-1)

Authors   A. Hannemann[1], N. Friedrich[1], J. Lüdemann[1], H. Völzke[2], R. Rettig[3], J. Peters[3], M. Reincke[4], A. Döring[5], M. Nauck[1], H. Wallaschofski[1]

Clinical Chemistry 57:11
1607—1611 (2011)

Reference Values for Aldosterone—Renin Ratios in Normotensive Individuals and Effect of Changes in Dietary Sodium Consumption

Michiel N. Kerstens,[1*] Anneke C. Muller Kobold,[2] Marcel Volmer,[2] Jan Koerts,[2] Wim J. Sluiter,[1] and Robin P.F. Dullaart[1]

Età:

20-54/55-74

Sesso

Maschi/Femmine

Aldosterone/renina: variabili preanalitiche

Review Article

Laboratory Investigation of Primary Aldosteronism

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*For correspondence: Prof Michael Stowasser at m.stowasser@uq.edu.au

Dieta sodica:
  Dieta libera: no restrizione sodica

Concentrazione del potassio:
  Ipopotassiemia associata con risultati falsi negativi: correzione
  Iperpotassiemia fattizia: corretto prelievo di plasma

Aldosterone/renina: variabili preanalitiche

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Farmaci
  Induttori di falsi positivi: beta-bloccanti, metilldopa, clonidina, FANS
  Induttori di falsi negativi: diuretici, calcio-antagonisti, ACE-inibitori, ARBs
  Effetti complessi: inibitori renina, estroprogestinici, antidepressivi

Aldosterone/renina: variabili analitiche

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Dosaggio dell'aldosterone (e della renina)

Metodi immunometrici isotopici vs. non-isotopici
Metodi manuali vs metodi automatizzati
Effettivo impiego dei metodi cromatografici

Aldosterone: analytical characteristics

Concentration (Plasma/serum): ng/L

(Cortisol/Aldosterone: 1000/1)

Methods of detection

Bioassay (isotope derivative assay)
Radioimmunoassay
GC-MS/MS
HPLC-MS/MS
Enzyme immunoassay (ELISA)
Chemiluminescence immunoassay

60 Years of aldosterone (electrocortin)
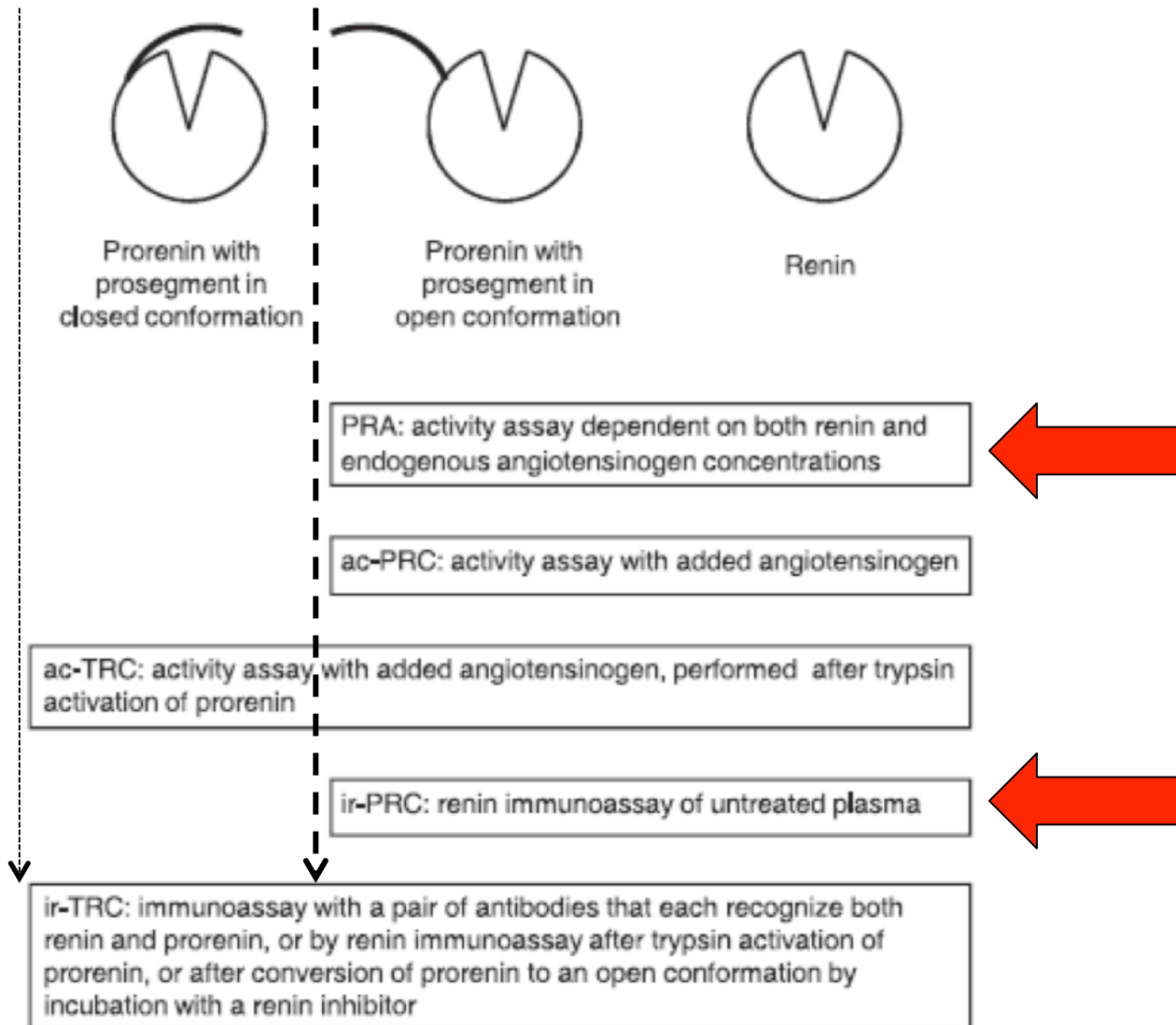**Simpson SA, Tait JF, Reichstein T, 1953**

Aldosterone: assay methods

Method	Tracer	Chromatography	Extraction	Procedure	Author
Bioassay	^{3}H, ^{14}C	Paper, Celite, Amberlite, Sephadex	Dichloromethane Benzene-methanol	Manual	Simpson, 1953
RIA	^{3}H	Paper, Celite, Amberlite, Sephadex	Dichloromethane Benzene-methanol	Manual	Mayes, 1970 Boyard, 1970
RIA	^{3}H	No	Dichloromethane Benzene-methanol	Manual	Drewes, 1973 Farmes, 1973
RIA	^{125}I	No	Dichloromethane Benzene-methanol	Manual	Hariyama, 1976
RIA	^{125}I	No	No	Manual	Al-Duijaili, 1978
GC-MS/MS	-	C18 column	No	Manual	Prone, 1988
HPLC-MS/MS	-	C18 column	Acetonitrile	Semiautomated	Fredline, 1997
CLIA	AE	No	No	Automated	Perschel, 2004

Prorenin with prosegment in closed conformation

Prorenin with prosegment in open conformation

Renin

PRA: activity assay dependent on both renin and endogenous angiotensinogen concentrations

ac-PRC: activity assay with added angiotensinogen

ac-TRC: activity assay with added angiotensinogen, performed after trypsin activation of prorenin

ir-PRC: renin immunoassay of untreated plasma

ir-TRC: immunoassay with a pair of antibodies that each recognize both renin and prorenin, or by renin immunoassay after trypsin activation of prorenin, or after conversion of prorenin to an open conformation by incubation with a renin inhibitor

Nomenclature for different forms of renin or prorenin measured by activity assays and immunoassays

Renin form measured	Activity assays	Immunoassays	Units
Plasma renin activity (PRA)	PRA: Ang I production by plasma renin activity (both renin or prorenin with open conformation) acting on endogenous plasma angiotensinogen		μg or nmol Ang I/L/h
Plasma renin concentration (PRC)	Ac-PRC: Ang I production by plasma renin enzymatic activity (both renin and prorenin with open conformation) acting on exogenous angiotensinogen	Ir-PRC: Renin immunoassay of untreated plasma (measures both renin and prorenin with open conformation)	IU/L, ng/L, or pmol/L

PRA vs PRC

Factor	PRA	PRC
Ice cooling	Yes	No
Angiotensinogen (liver cirrhosis, cardiac failure, pregnancy, glucocorticoid excess, estrogens, diabetes)	Dependent	Indipendent
Reproducibility	Poor	Acceptable
Sensitivity	High	Acceptable
Prorenin interference	No	No, with short incubation time at 37°C
Savings of time	No	Yes

Aldosterone: analytical variability

Test	Method	Concentration (mean)	Concentration (DS)	CV%	p
Nichols Advantge	CLIA	**150.0**	17.2	11.4	
DPC-Siemens Coat-A-count	RIA	**222.0**	96.1	**43.2**	0.001
Adaltis Maia	RIA	**239.5**	15.3	10.7	0.001
Demeditec	RIA	**351.4**	15.3	18.6	0.006

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Fisher E, et al. Horm Metab Res 2013;45:526-31

Renin: analytical variability

Test	Method	Concentration (mean)	Concentration (DS)	CV%	p
Nichols Advantge	PRC-ILMA	2.86	0.42	14.6	
CIS-bio	PRC-IRMA	4.48	0.69	15.4	0.001
Adaltis Maia	PRA-RIA	0.015	0.04	26.7	
DiaSorin	PRA-RIA	0.020	0.06	30.0	0.009

Assay Characteristics Influence the Aldosterone
to Renin Ratio as a Screening Tool for Primary
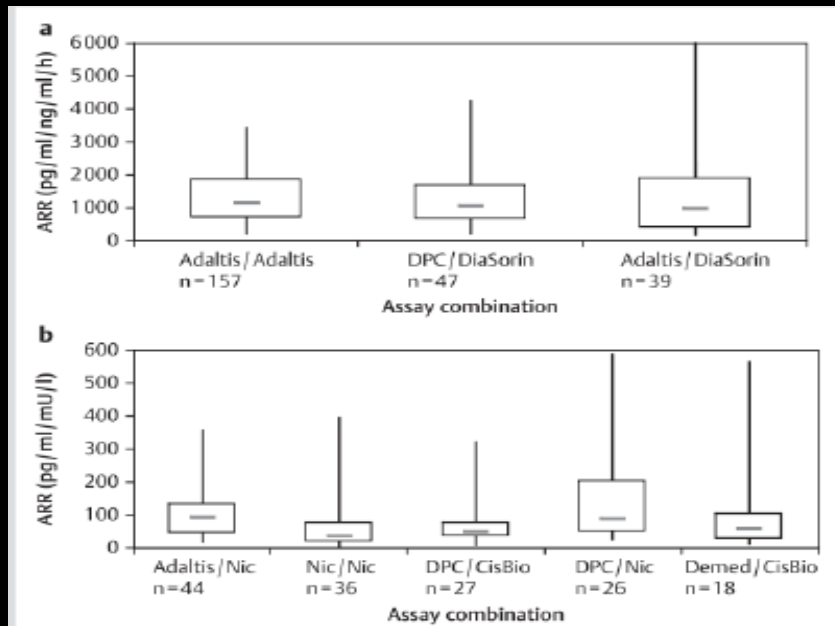Aldosteronism: Results of the German Conn's Registry

Authors   E. Fischer[1], S. Reuschl[1], M. Quinkler[2], L. C. Rump[3], S. Hahner[4], M. Bidlingmaier[1], M. Reincke[1]
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ARR: analytical variability

Assay Combination	ARR (mean)	p
Adaltis/Nichols	92.2	
DPC/Nichols	85.5	0.05
Demeditec/Cis-bio	57.1	0.01
DPC/Cis-bio	45.4	0.006
Nichols/Nichols	34.7	0.004

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		Unità
PAC	ng/dL	Convenzionali
	ng/L	Convenzionali
	pmol/L	SI
PRA	ng/mL/h	Convenzionali
	pmol/L/h	SI
PRC	ng/L	Convenzionali
	mU/L (IRP 68/356)	SI
ARR	**1/10/100**	

ARR: post-analytical variability

- Clinical sensitivity: 66-100%

- Clinical specificity: 61-96%

- Different cut-offs: 20-100

- Proposed cut-offs from Endocrine Society
  - PAC/PRA=60-80
  - PAC/PRC=91-122

- Sensitivity: 22.2%

- Specificity: 98.7%

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J. Hypertens 2013 (EAOP)

The automated CLIA analyzers: a new era ten years after?

Bari,
7-10 novembre 2013

Nichols Advantage

DiaSorin Liaison

IDS iSYS

Automated CLIA analyzers: analytical characteristics

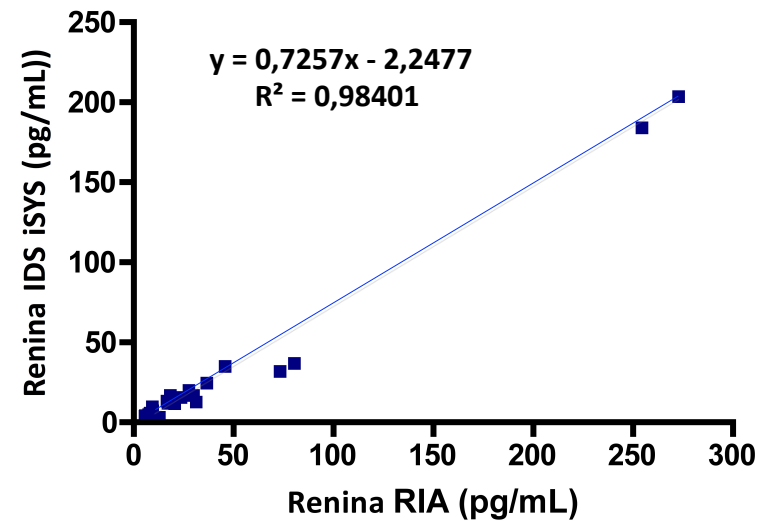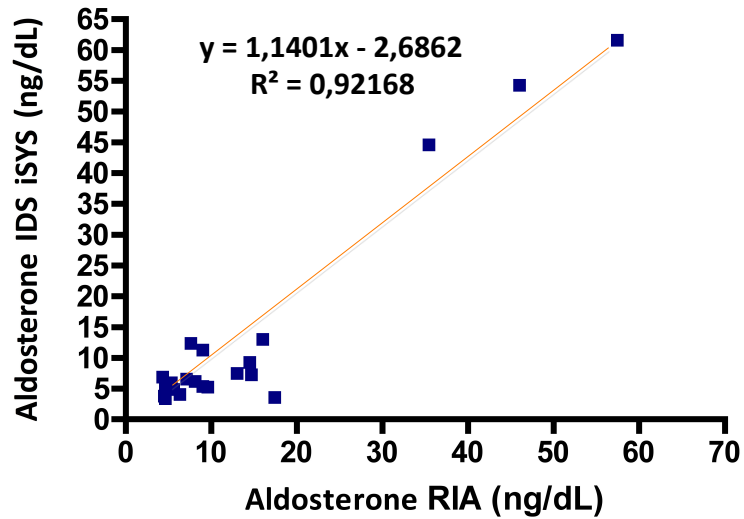| System	Precision WA (CV%)	Precision BA (CV%)	Sensitivity (FS)	Author (year)
Aldosterone (PAC)				
Manual RIA	3.5-6.4	3.6-10.4	2.0-3.0 ng/dL	Schirpenbach, 2006
Nichols Advantage	2.9-14.0	4.9-18.6	3.5 ng/dL	Diederich, 2007
DiaSorin Liaison	2.4-4.8	4-4-10.4	3.0 ng/dL	Package insert
IDS iSYS	1.8-9.0	1.9-9.2	3.7 ng/dL	Manolopoulou, 2012 Fortunato, 2012
Renin (PRC)				
Nichols Advantage	1.7-5.3	2.7-8.2	2.65 mU/L	Diederich, 2007
DiaSorin Liaison	1.3-7.2	7.3-10.4	5.0 mU/L	Dorrian, 2010
IDS iSYS	2.5-5.4	1.5-6.1	5.0 mU/L	Manolopoulou, 2012 Fortunato, 2012

Aldosterone-Renina IDS-iSYS

Parametri		Aldosterone
Sensibilità funzionale (LoQ)		6,8 ng/dL
Precisione	CV% Intra-assay	4%-10%
	CV% Inter-assay	2,3%-14%
Matrice del test		EDTA-Plasma
N° tests/ora		68 test/ora
Stabilità kit a bordo		1 mese
Stabilità calibrazione		4 giorni

Parametri		Renina
Sensibilità funzionale (LoQ)		2,1 mUI/L
Precisione	CV% Intra-assay	3%-11%
	CV% Inter-assay	2,3-11%
Matrice del test		EDTA-Plasma
N° tests/ora		68 test/ora
Stabilità kit a bordo		1 mese
Stabilità calibrazione		10 giorni

U-Aldosterone: è ancora utile?

- 9 lavori in letteratura negli ultimi 10 anni

- Impiego in medicina veterinaria, in pediatria e nel PA solo nel test di conferma con carico orale di sodio

- Criticità analitiche
    - Raccolta urine 24 ore: impiego di UACR
    - Presenza di aldosterone libero (5%), aldosterone-18 oxo-glucuronide (10%) renale, tetraidroaldosterone (35%), metaboliti non conosciuti (50%): estrazione o HPLC-MS/MS
    - Non può essere usato in: ipertensione severa non controllata, insufficienza renale, scompenso cardiaco, aritmie, ipopotassiemia

PA: test di conferma

Test	Sigla	Procedura	Analiti	Raccomandazioni
Carico orale di sodio	**SLT**	**>200mmol/die**	**UA**	**Young, Mulatero, ES**
Infusione di sodio	SIT	2 L 0.9% NaCl	PAC, PRA/PRC, K	Mulatero, ES, JES
Captopril challenge test	CCT	Captopril	PAC, PRA/PRC, Cortisolo	ES, JES
Test di soppressione con fludrocortisone	FST	Fludrocortisone	PAC, PRA/PRC, Cortisolo	Mulatero, ES
Furosemide upright test	FUT	Furosemide	PAC/PRA/PRC, Cortisolo	JES