Original Article

A screening procedure for primary aldosteronism based on the Diasorin Liaison® automated chemiluminescent immunoassay for direct renin

C A Dorrian[1], B J Toole[1], S Alvarez-Madrazo[2], A Kelly[1], J M C Connell[2] and A M Wallace[1]

[1]Department of Clinical Biochemistry, Macewen Building, Royal Infirmary, Glasgow G4 0SF; [2]BHF Glasgow Cardiovascular Research Centre, Faculty of Medicine, University of Glasgow, Glasgow G12 8TA, Scotland
Corresponding author: Mike Wallace. Email: alanm.wallace@nhs.net

Introduction

Recent guidelines[1] emphasize the importance of detecting the presence of primary aldosteronism (PA) as a secondary cause of hypertension because of the associated adverse cardiovascular risks and the availability of specific treatments. The prevalence of PA, previously thought to be as low as 1%, is now recognized as being 10% or higher in the hypertensive population.[1] This has led to a significant increase in the number of samples being sent for the determination of the aldosterone/renin ratio (ARR), which is accepted as the most reliable screening test currently available for this condition.[1]

The ARR is raised in PA and its diagnostic reliability depends upon the ability to measure plasma renin at extremely low concentrations. The traditional procedure involves incubating plasma with angiotensinogen (renin substrate), which generates angiotensin I. Angiotensin I is then measured by immunoassay and the plasma renin activity (PRA) calculated. This cascade has the advantage of being extremely sensitive, especially if the appropriate angiotensin I generation time is used. This is, however, a manual method that is time consuming and technically demanding with a low throughput. As demand for identifying patients with PA increases, a higher throughput, automated procedure would be advantageous.

Over the last few years, two methods have become commercially available for measuring renin concentration directly by immunoassay on automated platforms. The first was marketed by Nichols Diagnostics (San Juan Capistrano, CA, USA) for use on the Nichols Advantage® platform. Unfortunately, towards the end of 2006, just as detailed clinical evaluations of this method were beginning to appear,[2,3] the assay was withdrawn from the market. The second method for measurement of renin concentration, introduced more recently, is produced by Diasorin for use on the Liaison® platform. Both these systems are, or were,

based on the measurement of renin concentration directly by an automated immunochemiluminometric assay.

The current study was undertaken to evaluate the clinical use of the Diasorin direct renin assay on the Liaison® platform, as a replacement for the traditional manual PRA assay, with particular reference to screening for PA.

Methods

PRA was determined by radioimmunoassay of angiotensin I after generation from angiotensinogen by plasma renin (Renin MIAA, Adaltis Italia SpA, Bologna, Italy). Plasma renin concentration (PRC) was measured on the Diasorin Liaison® immunochemiluminometric analyser (DiaSorin Ltd, Wokingham, Berkshire, UK). Aldosterone was measured using a solid-phase (coated tube) radioimmunoassay kit (Siemens (UK) Ltd, Camberley, Surrey, UK). Human recombinant prorenin was purchased from Cayman Chemical (Ann Arbor, MI, USA [catalogue no. 10007599, >99% pure by sodium dodecyl sulphate polyacrylamide gel electrophoresis]).

Plasma prorenin was measured after proteolytic conversion to renin based on methodology described by Derkx *et al.*[4] This procedure required two aliquots of 0.5 mL potassium EDTA plasma. Aliquot 1 was used to determine the residual (basal) PRC, while aliquot 2 was subjected to proteolytic conversion of prorenin to renin using trypsin coupled to CH-Sepahrose 4B beads. Subsequently, both aliquots were analysed for PRC on the Liaison® platform. The concentration of endogenous prorenin was calculated by subtracting the renin measurement for aliquot 1 from that of aliquot 2.

Patients

A normotensive (BP < 130/85 mmHg) cohort ($n = 120$) was used for the determination of reference ranges. These adults (age 18–75 y) had no family history of hypertension, were not obese (BMI < 35 kg/m^2) and not on any medication. They were recruited from the local population as part of the control group for the MRC Bright study.[5] Approval for the study was granted by the local Ethics Committees and fully informed written consent from all of the participants was obtained. Anonymized routine patients' samples ($n = 266$) were used in the ARR method comparison and were from hypertensive patients suspected of suffering from primary or secondary aldosteronism. Samples were collected at least two hours after rising and then seated for at least 15 min prior to venepuncture. Any patient who attended our specialized hypertension clinic had hypokalaemia corrected prior to investigation. All subjects had unrestricted salt intake.

Sample collection and storage

Blood was collected into appropriate tubes (plain for aldosterone and potassium EDTA for renin) while subjects were upright. Samples were kept at room temperature and following centrifugation (3000 rpm, 10 min, 10°C) serum/plasma was aliquoted, snap frozen and stored at −70°C until analysis. For the control group, renin activity and renin concentration were measured simultaneously after being rapidly thawed to room temperature to avoid cryoactivation. For the patient group after initial routine analysis for aldosterone and renin activity, the samples were stored frozen at −20°C and renin concentration measured the second time the samples were subjected to a rapid thaw.

Results

Performance characteristics of the Liaison® direct renin assay

The analytical sensitivity, defined as the minimum detectable dose that can be distinguished by two standard deviations (SD) above zero, was 2.1 μIU/mL ($n = 20$). The functional sensitivity, defined as the lowest renin concentration detectable at which the coefficient of variation (CV) is 20%, was 5 μIU/mL. The assay working range is 5–500 μIU/mL, and samples containing renin concentrations above 500 μIU/mL were diluted in a diluent supplied by Diasorin (code – Endo 319133). Inter- and intra-assay variations were assessed using the two kit controls and two patient sample pools prepared 'in-house'. Intra-assay variation was less than 7.2% over the range 25–107 μIU/mL and inter-assay variation was less than 10.4% over the range 4.9–110 μIU/mL (Table 1).

Prorenin interference in PRC measurements

Cross-reactivity by prorenin in the Liaison® direct renin assay was evaluated using a commercial preparation of recombinant prorenin. The recombinant prorenin was added to seven human plasma samples, containing PRCs within the reference range, to give final recombinant prorenin concentrations of 0.5, 5 and 10 ng/mL. This resulted in an increase in PRC that was proportional to the amount of exogenous prorenin added (Figure 1a). Duplicate samples were also subjected to trypsin digestion and reanalysed for PRC (Figure 1b). A significant correlation ($r = 0.99$) between prorenin (after trypsin digestion) and PRC indicated equality of conversion of prorenin to renin across the range. The average proportion of prorenin measured before trypsin digestion compared with that after trypsin digestion was $9 \pm 4\%$ ($n = 21$).

Table 1 Intra- and interassay precison for the Diasorin Liaison renin concentration assay

Pool no.*	1	2	3	4
Intra-assay (n = 10)				
Mean (μIU/mL)	106.9	25		
CV (%)	1.3	7.2		
Inter-assay (n = 28)				
Mean (μIU/mL)	109.8	27.5	8.5	4.9
CV (%)	7.3	7.9	10.4	9.2

CV, coefficient of variation

*Plasma pools 1 and 2 were supplied with the kit; pools 3 and 4 were prepared 'in-house'

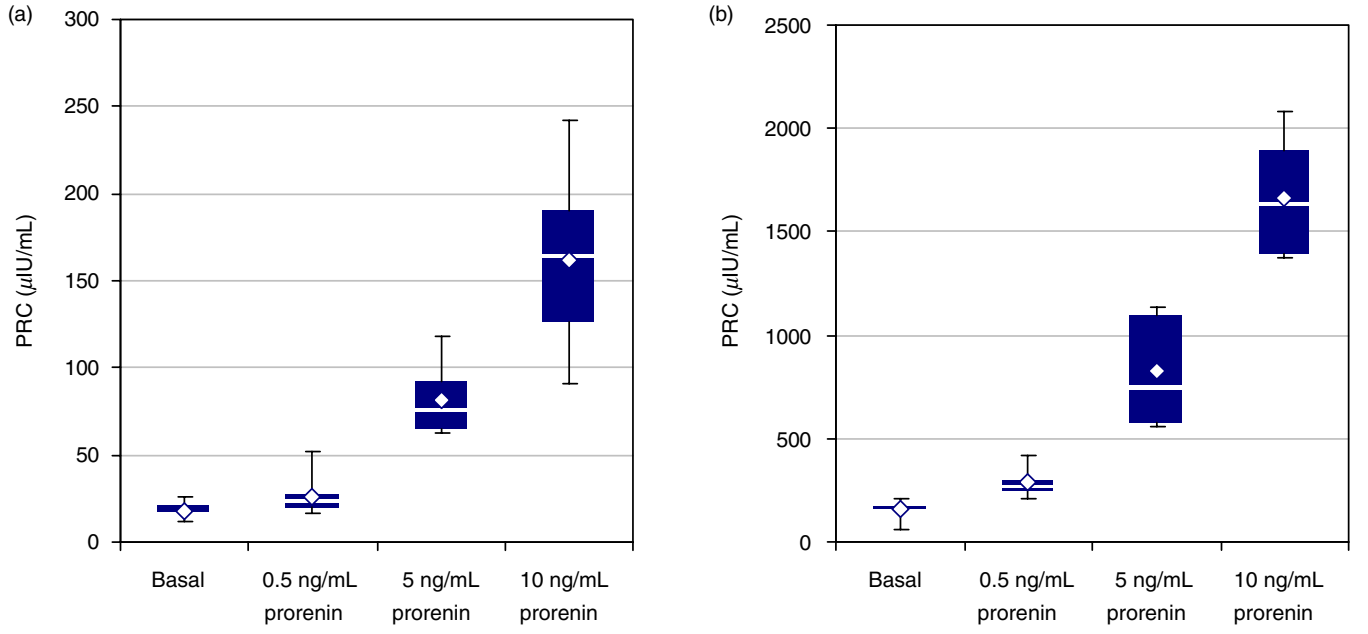

Figure 1 Recombinant prorenin (0.5–10 ng/mL) was added to seven plasma samples before (a) and after (b) tryptic digestion. Plasma renin concentration was measured using the Liaison® direct renin assay. The boxes show medians (◇) and upper and lower quartiles, while the whiskers indicate the minimum and maximum values

Reference ranges

To establish a reference range for PRA, PRC and prorenin, 95% confidence limits were calculated from measurements on 120 samples collected from normotensive (BP < 130/85 mmHg), non-obese (BMI < 35 kg/m^2) adults (age 18–75 y) who had no family history of hypertension and were not on any medication. Reference intervals obtained were <0.3–4.0 ng/mL/h for PRA, 5.0–44.9 μIU/mL for PRC and 44–423 μIU/mL for prorenin. The ARR reference intervals using aldosterone (pmol/L) with PRA and PRC were 38–587 pmol/L per ng/mL/h and 3–52 pmol/L per μIU/mL, respectively (Table 2).

Comparison of PRC with PRA measurements

Results obtained by the Diasorin® Liaison PRC method were compared with those obtained by the Adaltis® PRA assay. Both the control group and the routine patients' samples were used in this comparison ($n = 266$). Results below the PRA assay functional sensitivity (<0.3 ng/mL/h) were assigned the arbitrary figure of 0.2 ng/mL/h for comparative purposes and likewise PRC results below 5 μIU/mL were assigned the arbitrary figure of 4 μIU/mL.

Table 2 Reference ranges calculated from measurements on 120 samples collected from normotensive (BP < 130/85 mmHg) adults (age 18–75 y)

Measurement	Units	Range 2.5–97.5%
Aldosterone	pmol/L	<70–570
Renin activity (PRA)	ng/mL/h	<0.3–4.0
Renin concentration (PRC)	μIU/mL	5.0–44.9
Prorenin	μIU/mL	44–423
Aldo/PRA	pmol/L per ng/mL/h	38–587
Aldo/PRC	pmol/L per μIU/mL	3–52

Although the overall comparability appeared good ($r = 0.93$; $P < 0.05$; $n = 266$) (Figure 2), closer inspection revealed that comparability deteriorated when the renin activity fell below 1 ng/mL/h. For samples with PRA below 1 ng/mL/h, no significant correlation between PRA and PRC was identified (Figure 3).

Comparison of efficiency of aldosterone/PRC compared with aldosterone/PRA for screening purposes

Routine samples from hypertensive patients sent for investigation of possible aldosteronism were analysed using the Diasorin® Liaison PRC assay. The aldosterone/PRA and

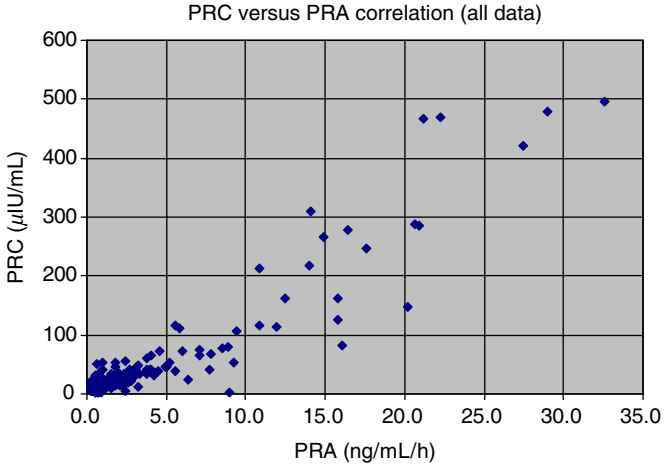

Figure 2 Comparison of PRC and PRA in hypertensive subjects being screened for aldosteronism ($r = 0.93$; $n = 266$). PRC, plasma renin concentration; PRA, plasma renin activity

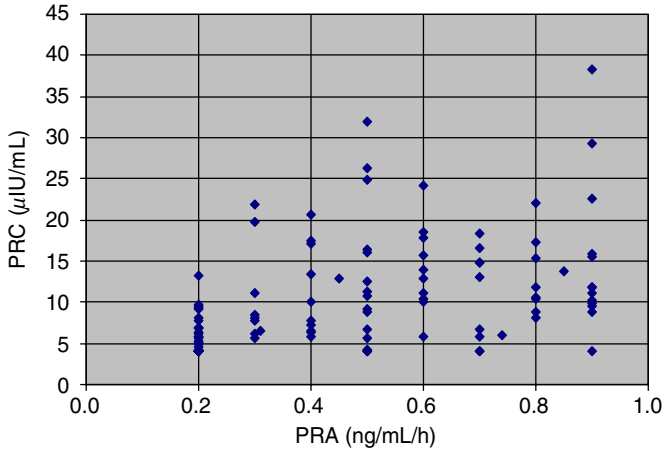

Figure 3 Comparison of PRC with PRA in hypertensive subjects being screened for aldosteronism with PRA < 1 ng/mL/h ($r = 0.14$; $n = 79$). PRC, plasma renin concentration; PRA, plasma renin activity

aldosterone/PRC ratios were calculated for each sample. Using the aldosterone/PRA ratio of >750 pmol/L as the gold standard in determining whether patients were recommended to undergo further testing for PA receiver operator characteristic (ROC) curves were calculated for different cut-off concentrations of the aldosterone/PRC ratio (Table 3). To achieve a sensitivity of 100% (i.e. no patients with potential Conn's syndrome being missed), the aldosterone/PRC ratio of >15 would have to be used, but specificity at this concentration was only 62%. To improve screening efficiency, the ROC curves were recalculated on the basis of using not only an aldosterone/PRA ratio of greater than or equal to 750 pmol/L as the deciding factor, but also the requirement that the aldosterone concentration was equal to or greater than 300 pmol/L (Table 4). The cut off of 300 pmol/L was selected as, in our experience, all cases of proven PA have an aldosterone well above this concentration at diagnosis. These criteria resulted in a sensitivity of 100% at an aldosterone/PRC cut off of 35, with the specificity much improved at 93%.

Two patients included in this comparison were subsequently proven to have PA. Their aldosterone/PRC ratios were 93 and 81, with plasma aldosterone/PRC concentrations of 1000 pmol/L/10.8 μIU/mL and 1200 pmol/L/14.8 μIU/mL, respectively.

Table 3 ROC analysis compared with current screen Aldo/PRA of >750 pmol/L

Aldosterone/PRC cut off	Sensitivity (%) 100% no false −ve	Specificity (%) 100% no false +ve
52	77	92
45	81	90
40	83	88
35	87	88
30	89	83
25	98	79
20	98	75
15	100	62

ROC, receiver operator characteristic; PRA, plasma renin activity

Table 4 ROC analysis compared with current screen Aldo/PRA of >750 pmol/L and aldosterone >300 pmol/L

A/PRC cut off	Sensitivity (%) 100% no false −ve	Specificity (%) 100% no false +ve
52	85	97
45	91	97
40	94	93
35	100	93
30	100	83
25	100	80
20	100	80
15	100	30

ROC, receiver operator characteristic; PRA, plasma renin activity

Discussion

Recent international guidelines on PA[1] recommend the use of the plasma ARR to detect cases of PA. These guidelines comment on technical aspects of renin measurement. As recommended in addition to kit controls to monitor precision of the PRC measurement, we also included plasma pools, prepared 'in- house', over the diagnostic range. We obtained within- and between-batch assay reproducibility (<7.2% and <10.4%, respectively), which was comparable with those described by the manufacturers in the kit insert (<5.6% and <12.8%, respectively). Given that the ARR is mathematically dependent on the method of renin measurement,[6] sensitivity is an issue. The guideline recommendation is that assay sensitivity should be as low as <0.3 ng/mL/h,[1] which by applying a conversion factor was calculated as 2 μIU/mL for direct measurement of renin concentration. Unfortunately, the recommendations do not state whether this is analytical or functional sensitivity. In the current study, we obtained analytical and functional sensitivities of 2.1 and 5 μIU/mL, respectively, values that are higher than reported in the manufactures kit insert (0.53 and 1.96 μIU/mL, respectively). This difference suggests that assay sensitivity may be dependent to some extent on kit lots and instrument performance. It is thus important that the upper limit of ARR calculated using PRC is set as low as possible to accommodate such changes in assay sensitivity and to avoid missing possible cases of PA.

In both PRA and PRC assays, there is a risk of interference due to cryoactivation of prorenin to renin. At temperatures around 6°C, prorenin can undergo a reversible conformational change which exposes the renin active site ('open' conformation).[7] In the current study, prior to and during measurement, temperatures for potential cryoactivation (4–6°C) were avoided.

The poor correlation between PRC and PRA observed at concentrations of PRA below 1 ng/mL/h led us to suspect that there may possibly be interference by prorenin in the measurement of renin using the PRC method. Indeed, addition of recombinant prorenin to plasma samples showed a significant 'cross-reactivity' of approximately 9%. Similar problems have previously been reported in the renin immunoradiometric assay (IRMA) described by Derkx[4] and in the Cisbio renin IRMA.[8] Any prorenin cross-reaction in the renin assay is a significant issue given that

prorenin concentrations in the peripheral circulation are of the order of 10 times higher than renin.[6,9] It is, however, also possible that the prorenin interference is indirect resulting from a conformational change within the added recombinant prorenin. At higher PRC concentrations the effect of prorenin interference, as a percentage of PRC, will be less marked accounting for the improved comparability between PRC and PRA at higher PRA concentrations >1 ng/mL/h.

The comparability of both types of renin measurement to screen for PA using the ARR was investigated. Using the PRA assay and a cut off of 750 pmol/L for the ARR as the 'gold standard',[1] we constructed ROC curves to identify the derived value that provided greatest sensitivity and specificity for the ARR calculated using the PRC assay. In this initial approach, we found that a calculated value of 15 provided sensitivity of 100%, but with an unacceptable concentration of specificity (62%). Adopting a more stringent screening approach using the PRA-derived ARR of >750 pmol/L, accompanied by an aldosterone value that was >300 pmol/L, we showed that the PRC method allowed us to identify an ARR value of >35 pmol/L/μIU/mL that offered sufficient sensitivity (100%) and specificity (93%) which is more acceptable for a screening procedure. This approach identified the same group of patients as the current screening test utilizing renin activity, but has the advantages of high throughput and economy. In addition two patients, subsequently identified as having PA by further testing, were identified among the routine samples screened. In both cases aldosterone was >300 pmol/L and the ARR was significantly raised using the PRC assay.

In summary, we emphasize that ARR is a screening and not a diagnostic test for PA. Currently, the only definitive diagnosis is retrospective after removal of a histologically proven adenoma and normalization of hypertension and/or hypokalaemia (if present). Recent evidence from population studies provided by our own group[10] and by others[11] suggest a progressive and positive relationship between ARR with blood pressure; a raised ARR is, however, not synonymous with PA. Given therefore that the ARR is a screening rather than a diagnostic tool, we compared the new PRC to our existing PRA procedure, as a measure of the renin component of the ARR, and showed that the same patient group was identified. This was despite concerns about possible cross-reaction of prorenin causing lack of apparent sensitivity at low PRCs. Once patients are identified by the ARR screening test, our routine policy is to perform a salt-loading test to further investigate the possibility of PA. We now routinely use PRC rather than PRA for both screening and subsequent detailed investigations.

DECLARATIONS

Competing interests: None.
Funding: This research was aided by funding from the British Heart Foundation.
Contributorship: All authors were involved in protocol development and data analaysis. CAD and AK were responsible for aldosterone and renin measurements; BT developed the prorenin method and performed all prorenin measurements; SA-M and JC organized patient selection; and CAD, BT and AMW prepared the manuscript. All authors approved the final version of the manuscript.
Acknowledgement: We are extremely grateful to Jim Smith for additional sample analysis.

REFERENCES

1 Funder JW, Carey RM, Fardella C, *et al.* Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 2008;**93**:3266–81
2 de Bruin RA, Bouhuizen A, Diederich S, Perschel FH, Boomsma F, Deinum J. Validation of a new automated renin assay. *Clin Chem* 2004;**50**:2111–6
3 Perschel FH, Schemer R, Seiler L, *et al.* Rapid screening test for primary hyperaldosteronism: ratio of plasma aldosterone to renin concentration determined by fully automated chemiluminescence immunoassays. *Clin Chem* 2004;**50**:1650–5
4 Derkx FH, Tan-Tjiong L, Wenting GJ, Boomsma F, Man in 't Veld AJ, Schalekamp MA. Asynchronous changes in prorenin and renin secretion after captopril in patients with renal artery stenosis. *Hypertension* 1983;**5**:244–56
5 Freel EM, Ingram M, Friel EC, *et al.* Phenotypic consequences of variation across the aldosterone synthase and 11-beta hydroxylase locus in a hypertensive cohort: data from the MRC BRIGHT Study. *Clin Endocrinol* 2007;**67**:832–8
6 Montori VM, Schwartz GL, Chapman AB, Boerwinkle E, Turner ST. Validity of the aldosterone–renin ratio used to screen for primary aldosteronism. *Mayo Clin Proc* 2001;**76**:877–82
7 Campbell DJ, Nussberger J, Stowasser M, *et al.* Activity assays and immunoassays for plasma renin and prorenin: information provided and precautions necessary for accurate measurement. *Clin Chem* 2009;**55**:867–77
8 Krop M, Garrelds IM, de Bruin RJ, *et al.* Aliskiren accumulates in renin secretory granules and binds plasma prorenin. *Hypertension* 2008;**52**:1076–83
9 Derkx FHM, Schalenkamp MADH. Human prorenin: pathophysiology and clinical implications. *Clin Exp Hypertens* 1988;**A10**:1213–25
10 Alvarez-Madrazo S, Padmanabhan S, Mayosi BM, *et al.* Familial and phenotypic associations of the aldosterone renin ratio. *J Clin Endocrinol Metab* 2009;**94**:4324–33
11 Meneton P, Galan P, Bertrais S, Heudes D, Hercberg S, Ménard J. High plasma aldosterone and low renin predict blood pressure increase and hypertension in middle-aged Caucasian populations. *J Hum Hypertens* 2008;**22**:550–8

(Accepted 7 January 2010)