assay (EIA)/Enzyme-Linked Immunosorbent Assay (ELISA)” by R.M. Lequin, recently published in Clinical Chemistry (1).

The assertion made in that article, that EIA/ELISA was first introduced, independently and simultaneously, by 2 scientific research groups, is correct but incomplete. In fact, a 3rd group, our team at the Pasteur Institute, also developed and published in 1971 an enzyme-immunologic method, this one for the measurement of serum IgG, with the aid of immunoadsorbants and enzyme-labeled antigens (2).

Incidentally, we also published in 1971 an immunoenzymatic method for the measurement of cellular immunoglobulins (3), although this method was not a classic (in the strictest sense), quantitative solid-phase immunoassay.

References

Rudolf M. Lequin
Diagnostic Consultancy Eindhoven
The Netherlands
Fax 31-40-290-8621
E-mail r.m.lequin@planet.nl

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Plasma Aldosterone Assays: Comparison between Chemiluminescence-Based and RIA Methods

To the Editor:
Measurement of plasma aldosterone is increasingly required in clinical practice because primary aldosteronism (PA) is the most frequent secondary cause of hypertension (1–4). Chemiluminescence-based (CL) assays are attractive alternatives to traditional RIAs for hormones such as aldosterone.

We compared aldosterone results obtained with a new CL method (Nichols Advantage) and an RIA [Sorin Biomedical Diagnostics (SBD)] in blood samples from 236 hypertensive patients. Blood samples were collected and immediately stored in separate aliquots at −20 °C until analysis. For 104 of the samples from these patients, we also compared the SBD-RIA results with results from a second RIA method [Maia Adalits (MA)]. We then used the CL assay and SBD-RIA to analyze samples obtained from 27 patients before and after inhibition testing (saline loading or fludrocortisone acetate) to confirm the diagnosis of PA. In all 27 patients, the inhibition test was performed because of a previous finding of an increased aldosterone-to-renin ratio (ARR).

Aldosterone was assayed in 2 aliquots from the same sample of each patient, and mean values were used for statistical analyses.

For the CL aldosterone assay, the mean within- and between-assay CVs were 2.6% and 4.7%, respectively, for samples between 213 and 1956 pmol/L. For the SBD-RIA method, within- and between-assay CVs were 3.8% and 3.6%, respectively, at a mean value of 371 pmol/L. For the MA-RIA method, within- and between-assay CVs were 3.5% and 4.1%, respectively, at a mean value of 845 pmol/L.

The correlation coefficient was 0.96 for both RIAs and 0.81 between the SBD-RIA and the CL assay; in the latter case, the coefficient decreased to 0.28 for the lowest values of the curve [aldosterone < 277.43 pmol/L (100 pg/mL)]. Passing–Bablok regression analysis of values obtained by both RIAs yielded a good slope [slope, 0.94; 95% confidence interval (CI), 0.88–0.99; intercept, 70.82 pmol/L (95% CI, 50.49–89.39 pmol/L); Fig. 1A]. The slope obtained for the comparison of the SBD-RIA and CL methods was less satisfactory [slope, 0.65 (95% CI, 0.61–0.68); intercept, −44.09 pmol/L (95% CI, −56.12 to −30.71 pmol/L; Fig. 1B], and was even poorer when aldosterone values
A intercept, 8.86 pmol/L (95% CI, 7.36–10.36); y = 0.94x + 70.82

B intercept, 8.86 pmol/L (95% CI, 7.36–10.36); y = 0.65x - 44.09

C y = 0.36x + 8.86

Fig. 1. Comparison of aldosterone assays by Passing-Bablok regression.

- Observed values; solid line, Deming prediction; dot-dashed line, bisector. The resulting regression equations are given in each panel. (A), comparison between the 2 RIA methods (n = 104); (B), comparison between the SBD-RIA and the CL assay in the total population (n = 236); (C), comparison between the SBD-RIA and the CL assay for RIA aldosterone values ≤277 pmol/L (100 pg/mL; n = 97).

<277.43 pmol/L were considered separately [slope, 0.35 (95% CI, 0.25–0.47); intercept, 8.86 pmol/L (95% CI, –6.71 to 21.11 pmol/L)].

Aldosterone values obtained by the CL assay were significantly lower than those obtained by the SBD-RIA (mean (SD), 223 (197) and 390 (268) pmol/L, respectively; P < 0.001, t-test for paired data).

In a case of suspected PA, an aldosterone value >416 pmol/L (150 pg/mL) increases the specificity and diagnostic accuracy of an increased ARR (2, 4). Moreover, to confirm the diagnosis, inadequate adrenal inhibition after volume expansion is required; i.e., patients are challenged by saline loading or fludrocortisoneacetate test (4). Failure of plasma aldosterone to decrease to <139 pmol/L (50 pg/mL) after challenge is regarded as diagnostic (4).

Confirmatory tests were administered to 27 patients characterized as having a high ARR. In all patients, aldosterone values before the inhibition test were >416 pmol/L when measured by the SBD-RIA, but only 9 pmol/L when measured by the CL method. Basal ARR was >38 pmol/mg (23 pg · mL⁻¹/po/mL), i.e., the cutoff used in our institution (3), in all 27 samples measured by SBD-RIA but in only 19 samples measured by the CL method. In 10 of 27 patients, PA could be confirmed (aldosterone values >139 pmol/L after inhibition) by RIA, but only 1 could be confirmed by the CL method.

From our comparison, it is clear that CL-based results do not correlate adequately with those obtained by SBD-RIA, with substantial underestimation at the limits of the reference intervals. The clinical consequences are potentially relevant for both screening and diagnosis of PA. For the CL assay, currently adopted cutoff values for ARR screening excluded a substantial proportion (35 of 51) of patients from further work up and from the correct diagnosis, compared with results obtained by RIA. Thus, the CL aldosterone assay showed very low sensitivity as a screening test.

Diagnosis of PA requires confirmation by a specific inhibitory challenge; in our series of inhibition tests, PA diagnosis was confirmed by the CL-based assay in only 1 of 10 patients with RIA-diagnosed PA. When we used the RIA results as the reference interval, the CL assay failed to confirm PA in 90% of cases, thus showing very low specificity.

Our data demonstrate that use of the CL assay without validation by an RIA method might lead to undiagnosed PA in a large number of patients.

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References


Francesca Pizzolo1*  
Angela Corgnati2  
Patrizia Guarini1  
Chiara Pavan1  
Antonella Bassi2  
Roberto Corrocher1  
Oliviero Olivieri1

1 Unit of Internal Medicine  
Department of Clinical and Experimental Medicine  
University of Verona  
Verona, Italy

2 Institute of Clinical Chemistry  
University of Verona  
Verona, Italy

* Address correspondence to this author at: Dipartimento Medicina Clinica e Sperimentale, Università di Verona, Policlinico Borgo Roma, 37134 Verona, Italy. Fax 39-45-580111; e-mail francesca.pizzolo@univr.it.

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