

## Aldosterone Assays: An Urgent Need for Improvement

It should not be forgotten that the concentration of aldosterone in plasma is only  $\sim 1$  thousandth that of cortisol. In this issue of *Clinical Chemistry*, Schirpenbach et al. (1) draw much-needed attention to current problems with measurement of aldosterone. The issue is extremely important in clinical practice after recognition that primary aldosteronism is a much more common cause of hypertension than previously thought (2–6). Consequently, we have seen a marked increase in screening for this disorder by measuring the ratio of aldosterone to renin (ARR) and by suppression testing to definitively confirm or exclude the diagnosis, both tests that critically depend on reliable aldosterone measurement. Because every hypertensive patient deserves testing to reliably diagnose or exclude this specifically treatable and sometimes curable disorder, the opportunities are tremendous for those marketing commercial aldosterone assay methods (already widely available), and even more so for automated aldosterone assays that offer simplicity, speed, and the opportunity to reduce technician time and/or numbers. Hence, in the midst of a revival in interest in primary aldosteronism and aldosterone measurement, there has been a recent move toward automation of the aldosterone assay, driven primarily by considerations of convenience, cost, or profit. By comparing 2 assay methods and 1 automated method with their more laborious but established in-house assay, which places accuracy and specificity over speed and simplicity, the authors provide a very valuable service (1). Importantly, the commercial assays were found wanting in several critical areas.

Schirpenbach et al. have demonstrated marked differences in mean values among the 4 different assay methods. High “*r*” values were obtained, but it is of course widely known that correlations between results achieved by 2 methods have little bearing on accuracy. Nevertheless, some authors still place emphasis on them, which is potentially confusing. Although “*r*” values seemed best at the lower end of the range, this was also where Bland-Altman plots showed the greatest percentage differences between commercial assay results and in-house assay results (often differing by 100% or more). Although actual numbers were not given, such differences would lead to appreciable numbers of patients being categorized differently as “positive” (primary aldosteronism confirmed or likely) or “negative” (primary aldosteronism excluded) depending on which assay method was used to measure postsaline (suppression test) aldosterone concentrations. Similar concerns obviously also apply to aldosterone/renin ratio testing, used to screen for primary aldosteronism and to select patients for suppression testing. Clearly this problem has serious implications for hypertension units attempting to identify patients with primary aldosteronism among hypertensive populations. For example, patients with primary aldosteronism might be missed, while others would be exposed to unnecessary saline loading.

The authors have been cautious to avoid over-interpreting their results. In particular, they have resisted the temptation to present false-positive and false-negative rates for saline infusion testing with each assay. There are good reasons for this decision. For one, there was no gold standard for the diagnosis of primary aldosteronism. Instead, several different criteria were used. Second, the infusion tests were performed while patients were still taking medications that may have affected results and rendered the tests unreliable. Mulatero et al. (7) have shown unequivocally that commonly used antihypertensive medications can lead to false-negative results in screening for primary aldosteronism. In practice, of course, avoiding the influence of antihypertensive drugs on aldosterone values is very difficult to achieve in the “resistant hypertension” group, in which the prevalence of primary aldosteronism has been reported to be as high as 20% (8). A 3rd reason relates to the difficult issue of selecting cutoff points for postsaline aldosterone concentrations. Should they have used a cutoff used by others (bearing in mind that there are several that have been recommended), or one derived from their own results? If the latter, from which of the 4 assays that they studied would the cutoff be derived, or would a different cutoff be used for each assay? They have correctly avoided suggesting that their in-house assay should have been regarded as the gold standard method, although they do point out that it was the only one that used an extraction step to minimize interference by other compounds. Further studies, taking more careful measures to avoid the effects of potentially interfering medications, would be required to compare different assays in terms of their accuracy in detecting and diagnosing this disorder.

Highly reproducible aldosterone and renin assays are essential not only for the detection and diagnosis of primary aldosteronism, but also for its ongoing management. The ARR appears to be more dependent on renin than aldosterone (9), especially when renin concentrations are low (as in patients with primary aldosteronism), in which case small absolute changes can result in large changes in the ARR. It could be argued, therefore, that for the purpose of ARR measurement in screening for primary aldosteronism, it is more important to measure renin accurately than aldosterone. However, false-positive and false-negative ARR values may also result from inaccurate aldosterone measurement. Furthermore, reliable quantification of aldosterone is critical during subsequent suppression testing (in which the definitive confirmation or exclusion of primary aldosteronism depends on the measured aldosterone concentration) and adrenal venous sampling, the results of which largely determine whether a patient is a candidate for unilateral adrenalectomy, or alternatively, treatment with aldosterone antagonist medication (10).

Because of the wide availability of commercial assays and the increasing demand associated with more widely

based screening for primary aldosteronism, the measurement of aldosterone and renin has moved increasingly from laboratories with meticulous quality control, clinical feedback, and long experience, to busy, shrinking-budget-driven general hospital laboratories or profit-driven private laboratories (11). Limited allocations for such specific purposes make it difficult for the all-purpose publicly-funded hospital or private enterprise general pathology laboratories to adopt the time-consuming and therefore expensive quality control practices followed by the best specialized unit laboratories, which go far above and beyond the quality control practices recommended in the commercial assay product insert or mandated by governmental rules such as those in CLIA. Although perhaps less vulnerable than renin assays, RIA analyses of plasma aldosterone performed with commercial assays are certainly not free from problems.

Over the past few years, faster, more convenient methods of directly measuring active renin (11, 12) and, more recently, aldosterone (13) with immunometric techniques and automated analyzers were rapidly adopted in large, busy laboratories. At the time of this writing, the major manufacturer of these types of assays (Nichols Institute Diagnostics) had unexpectedly ceased to provide reagents or further equipment, causing laboratories to return to manual RIAs; however, it is highly likely that automated methods will again be favored if reagents again become available that can be used on the Nichols Advantage analyzer or other automated platforms. Considerable work, involving cooperation between the manufacturers and the experts within the field, is required to validate and improve these methods before they can be accepted as sufficiently accurate to provide cutoff points for decisions on further work-up of patients possibly suffering from primary aldosteronism. In the study by Schirpenbach et al. (1), aldosterone concentrations measured by the Nichols automated method were below the assay's limit of detection for more than half the samples collected from healthy participants and nearly half those from patients with essential hypertension. Clearly this raises concerns about the potential for false-negative suppression test results associated with use of this assay. Our experience suggests also that the very rapid Nichols aldosterone assay has a serious problem with nonspecific interference, possibly because of the brevity of the "wash" immediately before chemiluminescence, leading to an unacceptably high blank value in bilaterally adrenalectomized and Addisonian patients. Weaknesses of the system (and areas for potential improvement) include the fact that it is calibrated by only a 2-point recalibration against a stored master calibration curve. We favor inclusion of plasma from bilaterally adrenalectomized or Addisonian patients as blanks and use of plasma pools from persons with known low, medium, or high values as calibrators. We believe that ongoing, in-depth evaluation of the automated systems is necessary.

Because of the critical role of validated assay techniques and the within-person biological variability of both aldosterone and renin, it is essential that patient management

decisions not be based on a single ratio. Before primary aldosteronism is determined to be highly likely or highly unlikely, the ratio should be repeated until it unmistakably is, or is not, raised, with medications and conditions of collection adjusted if indicated. The next step, a definitive test involving salt loading, is not entirely risk-free in patients with severe hypertension or compromised cardiac or renal function. A single measurement of ARR should never be relied upon (11).

Clearly, automated systems have appeal because of their speed and efficiency, the need for fewer staff, and the lower total cost, but at the end of the day, accuracy is the only consideration that really counts. Until accuracy can be guaranteed through careful and substantial analysis, reliance on these methods for making clinical decisions is premature. To achieve the necessary analytic and diagnostic accuracy would require not only preparation of certified reference materials (and ideally creation of an accepted reference method), but also general acceptance of a standard diagnostic test that would include compliance with a long list of considerations such as posture, time of day, and avoidance of interfering medications. Some of these considerations have been discussed previously (10, 11) and more recently by Giachetti et al. (14), but the issues are complex and a consensus will be difficult to reach. The US Endocrine Society considers achievement of a consensus of sufficient importance to have recently set up a task force with wide international representation to find a way forward.

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