Abstract—Direct renin concentration is replacing plasma renin activity in many laboratories for the investigation of primary aldosteronism, which may have a significant impact on the resulting aldosterone:renin ratios. We sought to develop a population-based approach to establishing an aldosterone:renin ratio cutoff when transitioning between assays. A population-based study was performed in Calgary, Alberta, Canada of 4301 individuals who received testing from January 2012 to November 2015. In 2014, direct renin concentration replaced plasma renin activity in routine testing. We described the prevalence of primary aldosteronism in our population before the change and, using the assumption of disease prevalence stability, determined the corresponding ratio cutoffs after the introduction of the new assay. During the initial portion of the study (using plasma renin activity), 4.9% of those screened were classified as highly probable cases, whereas 10.4% were considered probable and 28.9% possible using locally validated cutoffs. Aldosterone:renin ratio cutoffs were then determined for the direct renin concentration assay. A highly probable case of primary aldosteronism corresponded to a cutoff of >100 pmol L⁻¹ mIU⁻¹ L⁻¹ with hypokalemia. A probable case corresponded to a cutoff of >100 and a possible case to >35 pmol L⁻¹ mIU⁻¹ L⁻¹. In contrast, cutoffs derived using a conversion factor resulted in significantly higher cutoffs and the potential for missed cases. In conclusion, using large population data, historically consistent aldosterone:renin ratio cutoffs can be established when transitioning between assays. Population-derived cutoffs may be more appropriate for clinical use and less likely to result in false-negative classification than those obtained from conventional direct method comparisons. (Hypertension. 2017;69:450-456. DOI: 10.1161/HYPERTENSIONAHA.116.08407.) ● Online Data Supplement

Key Words: aldosterone ▪ hyperaldosteronism ▪ hypertension ▪ prevalence ▪ renin

Primary aldosteronism (PA) is a common form of remediable hypertension. The aldosterone:renin ratio (ARR) is the recommended screening test for PA in individuals with hypertension with or without hypokalemia. Elevations in the ratio are predominantly dependent on renin measurements, thus any alteration to the renin assay will have an impact on the resulting ARR.

Plasma renin activity (PRA) has traditionally been used to calculate the ARR; however, measurement of direct renin concentration (DRC) has become increasingly popular because the assay procedure is less labor and time intensive. Although numerous conversion factors have been proposed in an effort to generate clinically meaningful and stable cutoffs between assays, it remains uncertain whether an interchangeable relationship exists, especially as PRA and DRC are biologically distinct entities. Head-to-head comparisons are often performed by simultaneously measuring PRA and DRC, looking for correlations to establish stable conversion factors. Such studies are generally small thus limiting their general applicability. Moreover, the Endocrine Society has advised that the correlation between PRA and DRC is poor at lower renin levels, the domain of greatest importance for PA screening. Indeed, differences in analytical method can result in as much as a 2-fold variation in ARR cutoffs and classification error. On a population level, this may result in significant downstream consequences with tremendous clinical and public health implications.

Addressing this, we developed a novel yet easily generalizable approach to determining ARR cutoffs using population-based data without relying on direct assay comparisons or conversion factors. The specific aim of our study was to produce a new DRC-based ARR threshold consistent with an established PRA-based ARR cutoff, thus providing diagnostic congruity with established thresholds used in routine practice. Our approach is based on 2 inter-related assumptions: first, in a defined healthcare system with a relatively static population pool, screening is performed in similar types of patients; and second, of those who are screened, the prevalence of PA has remained stable over time.
Methods

This study was granted exemption by the University of Calgary Conjoint Health Research Ethics Board from formal institutional ethics review because it was principally conducted for laboratory quality assurance purposes.

Study Design and Data Source

We conducted a population-based study of individuals undergoing ARR testing in Calgary, Alberta, Canada. Data were collected from the Laboratory Information System at Calgary Laboratory Services (CLS), the sole supplier of laboratory services in the Southern Alberta region of Canada. Annual workload volumes at CLS approach 30 million tests, serving a population in excess of 1.4 million persons. Renin testing was performed by measuring PRA by radioimmunoassay until the fall of 2014 when the radioimmunoassay assay was replaced with an automated DRC assay. Measurement of DRC began on October 18, 2014, but testing for PRA continued until November 4, 2014. Thus, for a period of 18 days, there was an overlap between the 2 assays and the results were reported together.

Renin levels were acquired from January 2012 to November 2015 from CLS, including both activity- and concentration-based data for the same patient population. In addition, data for aldosterone, ARR, and plasma electrolytes were obtained. Clinical information on blood pressure, prescription drug dispensations, diagnostic codes, and physician encounters was not available. It should be noted that on August 24, 2014, CLS changed aldosterone assays from radioimmunoassay to chemiluminescent immunoassay, leading to a small positive analytical bias of 10% (Figure S1 in the online-only Data Supplement) which did not affect the calculated ARR during method validation (Figure S2). During the study period, there were no policy changes within CLS that may have potentially influenced the ordering behavior of physicians for aldosterone, renin, ARR, or electrolyte testing.

Biochemical Testing

Serum aldosterone was measured either by solid-phase radioimmunoassay using the Siemens Coat-A-Count Aldosterone assay (Siemens Healthcare Diagnostics, Tarrytown, NY) or by chemiluminescent immunoassay using the Diasorin Liaison XL platform (Diasorin, Mississauga, Ontario, Canada). Both assays exhibited imprecision levels <10% across the measuring range, with limits of detection of 70 and 50 pmol/L for the radioimmunoassay and chemiluminescent immunoassay methods, respectively. Importantly, this change in assay formulation had no effect on the overall diagnosis rates for PA. PRA was measured by radioimmunoassay with the GammaCoat Plasma Renin Activity 1st assay (Diasorin, Stillwater, MN). The lower reporting limit was 0.01 ng mL−1 h−1. DRC was measured using the Diasorin Liaison XL platform using a chemiluminescent immunoassay calibrated to the WHO International Standard 68/356. The lowest reporting limit of 1.0 mIU/L was used.

Case Definitions

The ARR was calculated by dividing aldosterone concentration (pmol/L) by the available renin value (ng mL−1 h−1 or mIU/L). An ARR threshold of >550 pmol L−1 ng−1 mL−1 h−1 based on PRA was considered elevated at our institution; its use and validation with clinical diagnoses and outcomes have been reported.13,14 Recognizing that the total number of people who received ARR testing, whereas only those who received both ARR and serum electrolyte testing were included in the highly probable category. Two sets of DRC-based ARR cutoffs were generated. The first was derived using population data. The prevalence of cases identified above, using PRA-based ARRs, was used to predict corresponding DRC-based ARR cutoffs. ARR cutoffs were selected in such a way that the measured prevalence would match historical values as closely as possible. The second set of DRC-based ARR cutoffs were generated using conventional direct method comparison. This was performed using independent samples from 40 individuals who received both PRA and DRC testing. Correlation was determined, and the slope of the best fit line was used as the conversion factor between the assays. The performance of the 2 sets of DRC-based ARR cutoffs was then evaluated. The y′ test was used to examine for changes in the prevalence of PA over time, according to 6-month intervals. Finally, to account for the possibility of selection bias in our primary analysis (where we only included the most recent ARR result if >1 ARR was collected during the study period), we performed a sensitivity analysis. Accordingly, the entire analysis was repeated by including the maximum and minimum ARR measurements. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Inc, Cary, NC).

Analyses

For individuals who received >1 ARR test, only the most recent result was included in our primary analysis because it was felt that the most proximate measurement would most likely reflect collection under optimal testing conditions. For the time period when ARRs were based on PRA measurements, the prevalence of PA was determined according to possible, probable, and highly probable categories. For the possible and probable prevalence calculations, the denominator was the total number of people who received ARR testing, whereas only those who received both ARR and serum electrolyte testing were included in the highly probable category. Two sets of DRC-based ARR cutoffs were generated. The first was derived using population data. The prevalence of cases identified above, using PRA-based ARRs, was used to predict corresponding DRC-based ARR cutoffs. ARR cutoffs were selected in such a way that the measured prevalence would match historical values as closely as possible. The second set of DRC-based ARR cutoffs were generated using conventional direct method comparison. This was performed using independent samples from 40 individuals who received both PRA and DRC testing. Correlation was determined, and the slope of the best fit line was used as the conversion factor between the assays. The performance of the 2 sets of DRC-based ARR cutoffs was then evaluated. The y′ test was used to examine for changes in the prevalence of PA over time, according to 6-month intervals. Finally, to account for the possibility of selection bias in our primary analysis (where we only included the most recent ARR result if >1 ARR was collected during the study period), we performed a sensitivity analysis. Accordingly, the entire analysis was repeated by including the maximum and minimum ARR measurements. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Inc, Cary, NC).

Results

Patient Characteristics

There were a total of 131483 laboratory measurements retrieved from our database for aldosterone, PRA, DRC, and electrolytes, corresponding to 4301 individuals over a 3-year period. Of these, there were 3126 ARRs based on PRAs and 1283 based on DRCs. Most individuals (91%) had accompanying electrolyte measurements (2738 for ARRs based on PRAs and 1177 for DRCs). The mean age was 51.7 (±19.4) years, and 47.0% were male.

Prevalence of Cases

A total of 4.9% of patients were classified as having highly probable PA by PRA, 10.4% were probable, and 28.9% were possible. The prevalence of these categories remained stable over time, with no significant change in the proportion of individuals with highly probable (P=0.63), probable (P=0.69), or possible (P=0.87) PA during the initial study period (Table 1).
after the introduction of the newly proposed cutoffs. There was no significant change in the proportion of highly probable ($P=0.87$), probable ($P=0.34$), or possible ($P=0.91$) cases over time (Figure).

### DRC-Based ARR Cutoffs Derived Using Direct Method Comparison

Direct method comparison was also conducted between the PRA and DRC assays using 40 independent patient samples (Figure S3). There was strong overall correlation ($R^2=0.85$). Using the line of best fit, we found that a PRA of 1 ng mL$^{-1}$ h$^{-1}$ converted to a DRC of 14.1 mIU/L. However, the correlation was not uniform particularly at lower renin levels. On applying the empirically derived conversion factor, we found that ARR cutoffs (based on PRA) of >550 and >2200 pmol L$^{-1}$ ng$^{-1}$ mL$^{-1}$ h$^{-1}$ corresponded to ARR values (based on DRC) of >39.0 and >156.0 pmol L$^{-1}$ mIU$^{-1}$ L$^{-1}$, respectively. We then evaluated the performance of these cutoffs in our population and found that 30 of 1177 (2.6%) would be classified as having highly probable PA, 78 of 1283 (6.1%) with probable PA, and 337 of 1283 (26.3%) with possible PA. When compared with the expected population prevalence from historical measurements (ie, 4.9%, 10.4%, and 28.9% for highly probable, probable, and possible PA, respectively), there was a significant difference in the number of highly probable ($P=0.04$) and probable ($P<0.001$) cases of PA though the number of possible cases remained similar ($P=0.62$).

### Sensitivity Analyses

A total of 463 (14.8%) individuals had multiple ARR measurements during the initial study interval. To address possible variability under different testing conditions, we conducted a sensitivity analysis by considering the maximum and minimum ratios from each patient with multiple ARR values. We found that the prevalence of highly probable PA ranged from 4.42% to 5.73%, probable cases from 9.02% to 12.73%, and possible cases from 25.27% to 31.86%. Accordingly, these values represent the possible extremes that may be expected with selection bias, but these results were broadly similar to those reported, and were still compatible with the findings from our main analysis with no significant differences in the proposed cutoffs.

### Table 1. Proportion of Unique Individuals With Different Categories of Biochemically Classified Primary Aldosteronism According to Aldosterone:Renin Ratios Using Plasma Renin Activity

<table>
<thead>
<tr>
<th>Dates</th>
<th>No. Tested for ARR</th>
<th>No. Tested for ARR and Electrolytes</th>
<th>No. With Highly Probable Diagnosis (%)</th>
<th>P Value †</th>
<th>No. With Probable Diagnosis (%)‡</th>
<th>P Value †</th>
<th>No. With Possible Diagnosis (%)‡</th>
<th>P Value †</th>
</tr>
</thead>
<tbody>
<tr>
<td>January 1 to June 30, 2012</td>
<td>515</td>
<td>483</td>
<td>18 (3.7%)</td>
<td></td>
<td>54 (10.5%)</td>
<td></td>
<td>152 (29.5%)</td>
<td></td>
</tr>
<tr>
<td>July 1 to December 31, 2012</td>
<td>429</td>
<td>391</td>
<td>23 (5.9%)</td>
<td>0.63</td>
<td>47 (11.0%)</td>
<td>0.69</td>
<td>123 (28.7%)</td>
<td></td>
</tr>
<tr>
<td>January 1 to June 30, 2013</td>
<td>504</td>
<td>446</td>
<td>24 (5.4%)</td>
<td></td>
<td>54 (10.7%)</td>
<td></td>
<td>146 (29.0%)</td>
<td></td>
</tr>
<tr>
<td>July 1 to December 31, 2013</td>
<td>590</td>
<td>521</td>
<td>27 (5.2%)</td>
<td></td>
<td>63 (10.7%)</td>
<td></td>
<td>173 (29.3%)</td>
<td></td>
</tr>
<tr>
<td>January 1 to June 30, 2014</td>
<td>708</td>
<td>579</td>
<td>29 (5.0%)</td>
<td></td>
<td>78 (11.0%)</td>
<td></td>
<td>210 (29.7%)</td>
<td></td>
</tr>
<tr>
<td>July 1 to November 4, 2014</td>
<td>380</td>
<td>318</td>
<td>12 (3.8%)</td>
<td></td>
<td>30 (7.9%)</td>
<td></td>
<td>99 (26.1%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3126</td>
<td>2738</td>
<td>133 (4.9%)</td>
<td></td>
<td>326 (10.4%)</td>
<td></td>
<td>903 (28.9%)</td>
<td></td>
</tr>
</tbody>
</table>

**Highly probable diagnosis defined by ARR >2200 pmol L$^{-1}$ ng$^{-1}$ mL$^{-1}$ h$^{-1}$ and serum potassium <3.3 mmol/L; probable diagnosis defined by ARR >550 pmol L$^{-1}$ ng$^{-1}$ mL$^{-1}$ h$^{-1}$; and possible diagnosis defined by ARR >550 pmol L$^{-1}$ ng$^{-1}$ mL$^{-1}$ h$^{-1}$. ARR indicates aldosterone:renin ratio.

*Denominator determined by total number of people receiving testing for ARR and electrolytes.

†Denominator comparing the frequency of cases over time per half-year interval.

‡Denominator determined by total number of people receiving testing for ARR.

Table 2. Historical and Proposed Aldosterone:Renin Ratio Cutoffs Before and After Implementation of the Direct Renin Concentration Assay

<table>
<thead>
<tr>
<th>Category</th>
<th>Current Case Detection Criteria Based on PRA Assay</th>
<th>Observed Prevalence With PRA Assay (No. of Cases/No. Tested)</th>
<th>Proposed Case Detection Criteria Based on DRC Assay</th>
<th>Corresponding Prevalence With DRC Assay (No. of Cases/No. Tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly probable diagnosis*</td>
<td>ARR &gt;2200 pmol L$^{-1}$ ng$^{-1}$ mL$^{-1}$ h$^{-1}$ and serum potassium &lt;3.3 mmol/L</td>
<td>4.9% (133/2738)</td>
<td>ARR &gt;100 pmol L$^{-1}$ mIU$^{-1}$ L$^{-1}$ and serum potassium &lt;3.3 mmol/L</td>
<td>4.7% (55/1177)</td>
</tr>
<tr>
<td>Probable diagnosis †</td>
<td>ARR &gt;2200 pmol L$^{-1}$ ng$^{-1}$ mL$^{-1}$ h$^{-1}$</td>
<td>10.4% (326/3126)</td>
<td>ARR &gt;100 pmol L$^{-1}$ mIU$^{-1}$ L$^{-1}$</td>
<td>11.2% (143/1283)</td>
</tr>
<tr>
<td>Possible diagnosis †</td>
<td>ARR &gt;550 pmol L$^{-1}$ ng$^{-1}$ mL$^{-1}$ h$^{-1}$</td>
<td>28.9% (903/3126)</td>
<td>ARR &gt;35 pmol L$^{-1}$ mIU$^{-1}$ L$^{-1}$</td>
<td>28.5% (366/1283)</td>
</tr>
</tbody>
</table>

ARR indicates aldosterone:renin ratio; DRC, direct renin concentration; and PRA, plasma renin activity.

*Denominator determined by total number of people receiving testing for ARR and electrolytes.

†Denominator determined by total number of people receiving testing for ARR.
Discussion

The ARR is the standard recommended screening test for PA and has been widely used since its introduction in 1981. However, as a screening test, the ARR is limited by a wide range of suggested cutoffs, intraindividual variation, and assay variability. The latter limitation has become an increasingly important consideration as DRC is progressively replacing PRA in routine use because of its increased convenience. Indeed, reference values for the ARR using DRC still remain incompletely defined, and selecting an appropriate institutional ARR cutoff with the introduction of a new assay can be difficult. Our study is the first to use a population-based approach to establish an ARR cutoff for use in screening, derived from a previously validated clinical and biochemical measure, and reflects the spectrum of PA. In our present study, we found the historical prevalence of PA to be 4.9% in our screened population of ≈3000 individuals using the PRA assay and found it to be stable over time. As informed by our population data, we were able to establish another ARR cutoff corresponding to the DRC assay to generate historically consistent results. In contrast, we found that using a conversion factor resulted in higher screening cutoffs, decreased sensitivity, and the potential for missed cases.

PRA and DRC are biologically distinct—the first reflecting the generation of angiotensin I from angiotensinogen, and the second being a direct measurement of renin concentration. To date, conventional methods used to correlate the 2 have uniformly involved direct head-to-head comparisons, typically in a small numbers of individuals. This approach has many limitations. First, although PRA and DRC are purported to be mathematically correlated, their relationship is not entirely linear, especially when renin levels are extremely high or low. This is particularly relevant when applied as a screening test for PA where renin is expected to be suppressed. Indeed, when we applied a conversion factor to our data, the resulting cutoffs resulted in a significant reduction in the number of detected highly probable and probable cases compared with what would be expected based on historical estimates. Although the number of possible cases remained similar, these were identified with lower ARR cutoffs and, therefore, less likely to be affected by extremely low renin levels. Importantly, we found that direct method comparisons may result in biased cutoffs, leading to unintentional changes in case definition and disease prevalence. Second, conversion factors are innately assay dependent and may not be generalizable to other laboratories. Third, and most importantly, it must be emphasized that the recommended screening test for PA is not renin itself, but rather the ratio between aldosterone and renin (ie, a transformed value). Notably, the relationship between renin and the resulting ARR is demonstrably nonlinear. As such, applying a conversion factor to renin, an individual component of a transformed value and expecting a
predictably proportionate change in the ratio is problematic. Illustrating this point, we found that a 4-fold increase in the ARR using the PRA assay (550–2200 pmol L−1 ng−1 mL−1 h−1) roughly corresponded to a 3-fold increase in the ARR derived from DRC (35–100 pmol L−1 mIU−1 L−1).

In contrast, there are several reasons why a population-based approach to defining an ARR cutoff is both intuitive and appealing. PA is not dichotomous but appears to exist along a continuum encompassing the spectrum of low-renin hypertension to overt hyperaldosteronism with hypokalemia. Accordingly, this condition is continuously distributed in the hypertensive population, but only those at the end of the distribution are usually labeled as having definite (or potentially surgical) disease, making the definition of aldosterone-mediated hypertension somewhat arbitrary. Variations in screening cutoffs, as such, are part of the reason for the range in the reported prevalence of PA in the literature. Reflecting the spectrum of disease, at our institution, we apply 3 biochemical definitions of disease when screening for PA, thereby enabling clinicians to interpret the ARR according to the pretest probability of disease and to help inform subsequent investigation or management. Ideally, ARR cutoffs should be determined according to individual laboratory and institutional validation. In a stable population, as long as the performance of a test remains the same—and the test continues to be applied to the same sorts of individuals—the detection rate should continue to be similar too. By using local data, institution-specific and locally validated cutoffs may be established rather than relying on externally derived conversion factors that may not be applicable to the local assay or population.

Despite the many strengths of our study (ie, a large cohort drawn from a well-defined geographic locale, complete capture of all blood tests performed in the population at a centralized laboratory, a demonstrably stable prevalence of biochemically defined cases of PA according to various thresholds of probability even after accounting for possible selection bias, and local estimates of disease prevalence that closely match those reported from other centers), there are some limitations. First, we defined the presence of PA solely by an elevated screening ratio. It is uncertain whether individuals who exceeded the cutoffs actually went on to confirmatory testing. Even so, our findings still remain valid as our principal aim was to establish a locally comparable for most drugs with the notable exception of direct renin inhibitors, but these are rarely used at our institution. Finally, the 2 key assumptions underlying our proposed approach are that ARRs have been ordered in a similar population of patients over time, and the prevalence of PA has remained stable in this population. Although we were able to demonstrate that the prevalence of biochemically defined PA remained stable and can attest that there were no known changes or interventions that may have impacted physician ordering behavior during the study period (either through local policy or national/international clinical practice guidelines), secular changes cannot be excluded. Longer term, clinically based internal studies are now needed to confirm the validity of these newly proposed ARR thresholds.

**Perspectives**

We described a novel, affordable, and generalizable approach to evaluating ARR cutoffs for screening of PA. Using population data, historically consistent thresholds can be established. Population-derived ARR cutoffs may be more appropriate for clinical use and less likely to result in false-negative classification than ARR cutoffs obtained from conventional direct method comparisons. There is potential for this approach to be extended to other conditions which likewise exist in a continuum in a population with a stable prevalence (ie, as is the case with most chronic diseases). Further research is needed to evaluate the performance of our strategy in other scenarios.

**Sources of Funding**

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**Disclosures**

None.

**References**

The diagnosis of primary aldosteronism: a debate on the scientific logic of continuum?


Kline


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**Novelty and Significance**

**What Is New?**
- The use of conversion factors to align aldosterone:renin ratios (ARRs) between laboratories may be unreliable because the correlation between plasma renin activity and direct renin concentration is poor at low renin levels. Alternatively, using population data, reliable ARR cutoffs can be generated when transitioning between laboratory assays.

**What Is Relevant?**
- Clinical practice guidelines recommend using conversion factors to align ARRs derived from different renin assays. The use of conversion factors may lead to biased ARR cutoffs with decreased sensitivity and increased false-negative misclassification.

**Summary**
Using population data, historically consistent and locally validated ARR cutoffs can be established when transitioning between renin assays.
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Online Supplement

A Novel Approach to Establishing an Aldosterone-to-Renin Ratio Cutoff for Primary Aldosteronism

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Correlation between plasma aldosterone concentration measurements using radioimmunoassay (gamma counter) and chemiluminescent immunoassay (Liaison XL) (panel A) with bias plot (panel B).
Correlation between the aldosterone-to-renin ratios generated using radioimmunoassay (gamma counter) and chemiluminescent immunoassay (Liaison XL) for plasma aldosterone concentration (panel A) with bias plot (panel B).
Correlation using direct method comparison between plasma renin activity (PRA) and direct renin concentration (DRC).