Validation of a New Automated Renin Assay

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Background: Measurement of plasma renin is important for the treatment of patients with congenital adrenal hyperplasia (CAH) and in the evaluation of patients with suspected hyperaldosteronism. Immunologic assays for plasma renin offer easier implementation and standardization than enzyme-kinetic assays for plasma renin activity, but their sensitivity and specificity have been questioned. We studied a renin immunochemiluminometric assay on an automated platform.

Methods: Renin was measured by an enzymatic assay, by IRMA, and by the new Nichols Advantage Specialty System immunochemiluminometric assay (ICMA), in plasmas from unselected individuals from our outpatient departments and in samples from patients with selected diagnoses.

Results: The detection limit in the ICMA was 0.1 mU/L. The recovery was >90%, and the imprecision (CV) was generally <9%. Mean (SD) concentrations measured by ICMA were 32 (21)% lower than those measured by IRMA. Renin concentrations as measured by ICMA were identical in serum and EDTA-, heparin-, and citrate-anticoagulated plasmas. Prolonged incubation of whole blood at room temperature before centrifugation did not affect renin concentrations. The central 95% interval for 80 healthy adults was 6–85.5 mU/L. Plasma renin as assessed by ICMA in patients with primary hyperaldosteronism was <0.2 mU/L.

Conclusions: The performance characteristics of the new renin ICMA allow its use for patients with CAH and for the diagnosis of mineralocorticoid hypertension. In view of the variability of renin concentrations, use

for other forms of hypertension or physiologic research calls for the development of uniform sampling protocols.

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Measurement of plasma renin content is generally thought to be the most practical method to determine the activity of the renin-angiotensin system. Previously, renin was measured by its enzymatic, angiotensin I-generating activity on its endogenous substrate, the so-called plasma renin activity (PRA).5 This assay is laborious and has poor intra- and interlaboratory reproducibility. This may be one of the reasons that renin measurements have been established only for the clinical diagnosis of primary hyperaldosteronism and the treatment of congenital adrenal hyperplasia (CAH).

For the past 15 years, direct measurement of renin by IRMAs has also been possible (1, 2). These IRMAs have several advantages over the enzymatic kinetic assays: they are less laborious and have the potential of better standardization because IRMAs are much less prone to interlaboratory variation. On the other hand, the specificity of the IRMA method has been questioned because the inactive precursor of renin, prorenin, may be converted on prolonged incubation at room temperature into a renin-like conformation that leads to overmeasurement of prorenin as renin (2, 3). This problem can be overcome by shorter incubation at a higher temperature (4).

The problem of overestimation of renin arises especially at low renin concentrations, which occur in states of salt loading and in primary hyperaldosteronism. Here the PRA method may have an advantage because low renin activity might still be measured by extending the incubation time. However, angiotensin I production in this assay is not linear over time, probably because of loss of angiotensin I on prolonged incubation (5). Here we report the performance of a new immunometric method on an

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Nonstandard abbreviations: PRA, plasma renin activity; CAH, congenital adrenal hyperplasia; NASS-ICMA, Nichols Advantage Specialty System immunochemiluminometric renin assay; and ND-IRMA, Nichols Diagnostics renin IRMA.
Materials and Methods

NICHOLS ADVANTAGE SPECIALTY SYSTEM FOR RENIN MEASUREMENT

The Nichols Advantage Specialty System (NASS) is an automated immunoanalyzer. The antigen of interest is sandwiched between a magnetic particle solid-phase capture antibody and an acridinium-labeled tag antibody. Quantification is by acridinium ester chemiluminescence detection for this immunochemiluminometric assay (ICMA). For the renin assay (NASS-ICMA), the solid-phase antibody is biotinylated R3-36-16, which is bound to streptavidin-coated magnetic particles during the incubation, and the second, acridinium-labeled antibody is R1-20-5. R3-36-16 recognizes both prorenin and renin, but R1-20-5 is specific for renin. Detailed information on the antibodies is given in Ref. (6). All materials for the NASS-ICMA were provided by the manufacturer. The instrument uses 200 μL of the plasma sample per test and requires a dead volume of 200 μL. Briefly, 200 μL of the plasma sample and the two anti-renin antibodies (30 μL each) are added to a disposable cuvette and incubated for 20 min at 37 °C. Afterward, 20 μL of the magnetic particles (suspending in phosphate-buffered saline, <0.95 g/L sodium azide, and ProClin-300) and 50 μL of the assay buffer (normal saline, sheep serum, <0.95 g/L sodium azide, and ProClin-300) are added and incubated at 37 °C for another 10 min. After a washing step, the acidic (hydrogen peroxide and nitric acid, both in a final concentration of 10 mL/L) and alkaline (sodium hydroxide in a final concentration of 10–30 g/L) trigger solutions are injected into the cuvette. The emitted light, expressed in relative light units, is measured by the integrated system luminometer and is directly proportional to the concentration of renin (mU/L). The system is calibrated by a two-point recalibration against a stored master curve. First results are available after 40 min (20 + 10 min of incubation and 10 min of pipetting, transport, and measuring steps), and the throughput is 85 results per hour. The maximum loading capacity of the Advantage system is 120 samples.

IRMA

The IRMA for renin (ND-IRMA) was purchased from Nichols Diagnostics and was performed as described by Deinum et al. (4). This assay uses the same antibodies as the Advantage specialty system. Measurements were performed in duplicate. To 200-μL aliquots of untreated plasma or renin calibrators we added 100 μL of a 1:1 mixture of biotinylated monoclonal antibody R3-36-16 (0.5 mg/L) and radiolabeled (~250 000 cpm) monoclonal antibody R1-20-5. This was followed by incubation of this mixture with an avidin-coated polystyrene bead for 6 h at 37 °C (IRMA37 °C, 6h). This incubation minimizes comeasurement of prorenin as renin (4). After incubation, the beads were washed three times and transferred to a clean tube. Radioactivity of bound antibody was counted for 5 min in a gamma counter. In our hands, this assay has a detection limit of 1.3 mU/L and a recovery and imprecision (CV) generally >90% and <10%, respectively (4).

PRA

For the PRA assay we used a modification of the method proposed by Sealey (7). The method includes blank subtraction, and the 18-h incubation step for low renin samples is eliminated. In this assay, 50 μL of maleic acid (pH 5.7) and 12.5 μL of a protease inhibitor solution consisting of 1 volume of 0.287 mol/L phenylmethylsulfonyl fluoride in ethanol, 2 volumes of 0.5 mol/L disodium EDTA, and 2 volumes of 100 g/L neomycin sulfate were added to 0.5 mL of plasma. The mixture was then incubated at 37 °C for at least two time periods between 0.5 and 3 h to check for linear angiotensin I generation. The generated angiotensin I was quantified by RIA (8). Results are expressed as nmol · L\(^{-1}\) · h\(^{-1}\). The lower limit of detection was 0.08 nmol angiotensin I · L\(^{-1}\) · h\(^{-1}\).

Performance Studies

To carry out our performance studies for the NASS-ICMA, we used plasma samples selected on the basis of their renin concentration and stock volume.

Participants

Samples were from unselected individuals recruited from our outpatient hypertension clinic. In addition, samples were studied from patients with specific diagnoses, such as patients with diabetes (n = 17; age range, 19–50 years), patients with renal artery stenosis (not on beta-blockers or angiotensin-converting enzyme inhibitors; n = 9; age range, 48–66 years), from pregnant women with (n = 11; age range, 24–39 years) or without (n = 11; age range, 20–32 years) preeclampsia, and from women on oral contraceptives (n = 7; age range, 24–37 years). Renin was measured for diagnostic purposes in three individuals (32, 62, and 60 years of age) with primary hyperaldosteronism. Renin was also measured in 14 children and young adults with CAH (age range 4–20 years) to titrate mineralocorticoid dose. To determine the central 95% interval, we studied 80 individuals (age range, 16–75 years; 25 males) with normal blood pressure and without medication or specified salt intake. All participants gave informed consent, and procedures observed the rules imposed by the Helsinki Declaration. Blood was taken from an indwelling venous catheter after the individual had been in the supine position for at least 45 min. The normotensive controls were sampled when they were in a seated position. Anticoagulation was with citrate (0.2 mL of 0.646 mol/L per 10 mL of blood) or, for sample type
comparison with plasma, in heparin- or EDTA-containing tubes (Vacutainer, BD Pre-analytical Solutions).

Results

**Performance of the NASS-ICMA**

**Sensitivity.** The limits of detection, defined as the mean result of the assay plus 3 SD in two series of 20 runs of the sample diluent, were 0.013 and 0.094 mU/L, respectively. The functional sensitivity, defined as the lowest renin concentration at which the CV in four series of five assay runs was <20%, was 2.65 mU/L (not shown). Although no official conversion factor between U and moles for renin has been established, this amounts to a functional sensitivity of ~65 amol/L (1 mU = 1 pg of renin of Mr 40 000).

**Precision.** The intraassay variation of three samples with various renin concentrations ranged from 1.7% to 5.3%. Interassay variation in seven samples with renin concentrations from 10 to 466.5 mU/L was 2.7–8.2% (Table 1).

**Linearity.** Dilution of four plasma samples with the sample diluent gave parallel lines whose slopes did not differ (Fig. 1). Measurement of renin in two series of mixtures prepared from two plasma samples with low and high renin content, respectively, yielded renin values that did not differ from expected values (Table 2).

**Method Comparison**

Plasma samples from 102 individuals were assessed for renin concentration by ND-IRMA and by NASS-ICMA and for PRA by enzyme kinetic assay (Figs. 2 and 3). The rank correlation coefficient for the PRA and NASS-ICMA was 0.88 (95% confidence interval, 0.83–0.92) and for the ND-IRMA and NASS-ICMA was 0.92 (0.89–0.95). Bland–Altman analysis (not shown) showed that the results obtained with the NASS-ICMA were 32% lower than those obtained with the ND-IRMA over the entire range of values. The NASS-ICMA results for controls and specific patient groups are shown in Fig. 4. As expected, renin values were low in three patients with primary hyperaldosteronism (0.01, 0.01, and 0.02 mU/L, respectively) and highly increased in patients with heart failure. The central 95% interval for healthy adults, sampled in the sitting position, was 6–85.5 mU/L.

**Stability**

In whole blood left at room temperature, the plasma renin content remained stable for a period of 72 h (Fig. 5). Stability was less if blood was centrifuged immediately and serum was left at room temperature.

**Sample Type Comparison**

Measurement of simultaneously taken plasma and serum samples yielded comparable results, although variance

### Table 1. Intra- and interassay CVs for the NASS-ICMA.

<table>
<thead>
<tr>
<th>Renin concentration, mU/L</th>
<th>Intraassay</th>
<th>Interassay</th>
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</thead>
<tbody>
<tr>
<td>10.0</td>
<td>ND</td>
<td>8.2 (n = 12)</td>
</tr>
<tr>
<td>19.0</td>
<td>ND</td>
<td>7.5 (n = 12)</td>
</tr>
<tr>
<td>31.6</td>
<td>5.3 (n = 20)</td>
<td>3.5 (n = 20)</td>
</tr>
<tr>
<td>70.9</td>
<td>ND</td>
<td>6.2 (n = 12)</td>
</tr>
<tr>
<td>108.5</td>
<td>1.7 (n = 15)</td>
<td>2.7 (n = 20)</td>
</tr>
<tr>
<td>241.5</td>
<td>1.8 (n = 10)</td>
<td>2.8 (n = 20)</td>
</tr>
<tr>
<td>466.5</td>
<td>ND</td>
<td>3.3 (n = 12)</td>
</tr>
</tbody>
</table>

* ND, not determined.

### Table 2. Recovery of renin in two plasma samples.*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Expected value, mU/L</th>
<th>Observed value, mU/L</th>
<th>Percentage of expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>336</td>
<td>317</td>
<td>94</td>
</tr>
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</tr>
<tr>
<td>II</td>
<td>101</td>
<td>98</td>
<td>97</td>
</tr>
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</table>

* In experiment I, a plasma containing 30.3 mU/L renin was mixed with a plasma pool containing 387.2 mU/L, and in II, a plasma with 59.4 mU/L renin was mixed with a plasma pool containing 350 mU/L. The volume-to-volume ratios in both experiments were 1:6, 2:4, 1:1, 4:2, and 6:1, respectively.
increased at very low concentrations (Fig. 6). This indicates that the NASS-ICMA provides similar results with serum and plasma, the latter irrespective of type of anticoagulant.

**Discussion**

Previously, we demonstrated that renin could be reliably measured with an IRMA (ND-IRMA) that used the same antibodies as the NASS-ICMA (4). One prerequisite was that the incubation was performed only for 6 h and at 37 °C to prevent a slow room-temperature-induced conformational change in prorenin, the enzymatically inactive precursor of renin, which would cause prorenin to be measured as renin. Theoretically, the risk of co-measurement of prorenin as renin in the NASS-ICMA is very low because of the very short processing time (30-min incubation at 37 °C and 10 min for transport and measurement). This is confirmed by the good agreement between the assay results obtained with the NASS-ICMA and the ND-IRMA. The NASS-IRMA is therefore useful for clinical management of patients with CAH and for the diagnosis of primary hyperaldosteronism. The sample-processing procedure is not critical, as evidenced by the stability of the result after prolonged storage of whole blood at room temperature. The NASS-ICMA yields iden-
tual results for serum and plasma. The sensitivity and linearity of the NASS-ICMA are excellent.

Of some concern may be the observation that the NASS-ICMA yielded lower values than the ND-IRMA. This may be caused by use of a different reference sample, but we did not evaluate the cause of this discrepancy because it was consistent over the entire range of renin values. Moreover, it is not the major obstacle to interlaboratory comparison of ICMA results at this moment; uniform sampling conditions are more important (see below).

The NASS-ICMA also correlated well with PRA, although this does not mean that it can replace the PRA assay in all circumstances. For example, oral contraceptives lower plasma renin concentrations (see Fig. 4) but generally do not influence PRA (9). This divergence between plasma renin concentration and PRA is explained by the large increase in angiotensinogen during contraceptive use. Because the PRA assay is based on the action of renin on endogenous angiotensinogen, which usually circulates at a concentration close to the $K_{mr}$ the increase in angiotensinogen and the decrease in renin during contraceptive use appear to balance out. This suggests that the PRA is regulated and that with changes in angiotensinogen concentration, such as during contraceptive use or pregnancy (9), the PRA may be more relevant physiologically.

Immunologic renin assays have better potential for international standardization than do PRA methods (10). This standardization is much in need because its lack has seriously hampered use of renin measurements in clinical situations and also makes the contribution of plasma renin to disease difficult to study (11). For example, it has been claimed that the PRA assay can be used as an aid for making therapeutic choices in hypertension (12). Although this concept is attractive, the development of guidelines based on PRA results has not been possible, undoubtedly in part because of the considerable inter- and intralaboratory variation for the PRA assay (10).

Renin immunometric assays have several properties that may lead them to become the standard method in cases where angiotensinogen concentrations are normal and the renin concentration and PRA correlate well. They can be calibrated against an international reference standard. The NASS-ICMA for renin may have additional advantages over the ND-IRMA in that the assay cartridges are prepared centrally and the assay is executed on a single type of automated instrument. The latter ensures uniform procedures and permits high-throughput handling.

There are two issues that need to be addressed. The first issue involves the establishment of a calibrator for renin immunoassays. We found a consistent proportional difference between the results obtained with the IRMA and the ICMA, which suggests that both assays use a different calibrator. The second, more important issue is the blood sampling protocol. Renin concentrations vary with posture, activity, and medication use (13); therefore, interlaboratory comparisons (or establishment of universal reference values) may not be possible when sampling conditions differ. In our own laboratory, we have individuals rest for 45 min, but in an outpatient clinic this may not be practical. It is therefore highly recommended that laboratories adopt standardized blood-sampling protocols. With these protocols, reference values can be developed for healthy and hypertensive individuals that may serve in the diagnosis and treatment of patients with CAH and hypertension.

In summary, the new rapid renin NASS-ICMA has a performance that at least equals that of an optimized ND-IRMA. The ICMA is easy to use and seems suitable for use in the clinical setting as well as for physiology studies.

Nichols Institute Diagnostika GmbH (Bad Vilbel, Germany) made available the Nichols Advantage Specialty System.

References