18-Hydroxycorticosterone, 18-Hydroxycortisol, and 18-Oxocortisol in the Diagnosis of Primary Aldosteronism and Its Subtypes

Paolo Mulatero,* Stefania Morra di Cella,* Silvia Monticone, Domenica Schiavone, Maria Manzo, Giulio Mengozzi, Franco Rabbia, Massimo Terzolo, Elise P. Gomez-Sanchez, Celso E. Gomez-Sanchez, and Franco Veglio

Department of Medicine and Experimental Oncology (P.M., S.M.d.C., S.M., D.S., F.R., F.V.), Division of Internal Medicine and Hypertension Unit, University of Torino, 10126 Torino, Italy; Clinical Chemistry Laboratory (M.M., G.M.), San Giovanni Battista University Hospital, 10126 Torino, Italy; Department of Clinical and Biological Sciences (M.T.), Division of Internal Medicine 1, University of Torino, San Luigi Hospital, 10043 Orbassano, Italy; and Division of Endocrinology (E.P.G.-S., C.E.G.-S.), G.V. (Sonny) Montgomery VA Medical Center and University of Mississippi Medical Center, Jackson, Mississippi 39216

Context: Diagnosis of primary aldosteronism (PA) is made by screening, confirmation testing, and subtype diagnosis (computed tomography scan and adrenal vein sampling). However, some tests are costly and unavailable in most hospitals.

Objective: The aim of the study was to evaluate the role of serum 18-hydroxycorticosterone (s18OHB), urinary and serum 18-hydroxycortisol (u- and s18OHF), and urinary and serum 18-oxocortisol (u- and s18oxoF) in the diagnosis of PA and its subtypes, aldosterone-producing adenoma (APA) and bilateral adrenal hyperplasia (BAH).

Patients: The study included 62 patients with low-renin essential hypertension (EH), 81 patients with PA (20 APA, 61 BAH), 24 patients with glucocorticoid-remediable aldosteronism, 16 patients with adrenal incidentaloma, and 30 normotensives.

Intervention and Main Outcome Measures: We measured s18OHB, s18OHF, and s18oxoF before and after saline load test (SLT) and 24-h u18OHF and u18oxoF.

Results: PA patients displayed significantly higher levels of s18OHB, u18OHF, and u18oxoF compared to EH and normal subjects; APA patients displayed s18OHB, u18OHF, and u18oxoF levels significantly higher than BAH patients. Similar results were obtained for s18OHF and s18oxoF. SLT significantly reduced s18OHB, s18OHF, and s18oxoF in all groups, but steroid reduction was much less for APA patients compared to BAH and EH. The s18OHB/aldosterone ratio after SLT more than doubled in EH but remained unchanged in APA patients.

Conclusions: u18OHF, u18oxoF, and s18OHB measurements in patients with a positive aldosterone/plasma renin activity ratio correlate with confirmatory tests and adrenal vein sampling in PA patients. If verified, these steroid assays would refine the diagnostic workup for PA. **(*J Clin Endocrinol Metab* 97: 881–889, 2012)**

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* P.M. and S.M.d.C. contributed equally to this study.

Abbreviations: A/C, Aldosterone/cortisol ratio; AII, angiotensin II; APA, aldosterone-producing adenoma; ARR, aldosterone/PRA ratio; AVS, adrenal vein sampling; BAH, bilateral adrenal hyperplasia; CT, computed tomography; EH, essential hypertension; GRA, glucocorticoid-remediable aldosteronism; LREH, low-renin essential hypertension; MR, mineralocorticoid receptor; NSAT, nonsecreting cortical adrenal tumor; 18OHB, 18-hydroxycorticosterone; 18OHF, 18-hydroxycortisol; 18oxoF, 18-oxocortisol; PA, primary aldosteronism; PRA, plasma renin activity; ROC, receiver operator characteristic; SLT, saline load test.

Primary aldosteronism (PA) is the most common form of secondary hypertension (1). The detection of primary aldosteronism is of particular importance, not only because it provides an opportunity for targeted treatment, either surgical for aldosterone-producing adenomas (APA) or medical with mineralocorticoid receptor (MR) antagonists for bilateral adrenal hyperplasia (BAH) (2), but also because it has been extensively demonstrated that patients affected by PA are more prone to cardiovascular events and target organ damage than essential hypertensives (3, 4). According to The Endocrine Society Guidelines, the diagnosis of PA is made following a three-step procedure comprising screening, confirmation/exclusion testing, and subtype diagnosis (1).

The most common screening test for PA is the measurement of the aldosterone/plasma renin activity (PRA) ratio (ARR); a positive ARR is followed by a confirmatory test, preferably using a saline load, to definitively confirm/exclude PA (1, 2). This is necessary to spare individuals with a falsely positive ARR costly and invasive subtype diagnostic procedures. However, there is no agreement on which of four accepted confirmatory tests should be performed because each test displays both advantages and potential pitfalls (5). Adrenal vein sampling (AVS) is the only reliable method to differentiate unilateral from bilateral PA (1, 6). Unfortunately, this test is costly and requires a dedicated and expert radiologist, and thus, it is not available in most hospitals.

18-Hydroxycorticosterone (18OHB) is an intermediate precursor in aldosterone biosynthesis with low affinity for the MR that originates from the conversion of corticosterone by the aldosterone synthase (7), although small amounts may be produced by the 11β-hydroxylase (8). Serum concentrations of 18OHB increase with increased aldosterone synthesis due to sodium depletion and angiotensin II (AII) infusion (9). It has been suggested that 18OHB levels may be useful for the differential diagnosis between BAH and APA because it is produced in higher relative concentrations in APA (10).

18-Hydroxycortisol (18OHF) and 18-oxocortisol (18oxoF) are known as "hybrid steroids" because they have structural characteristics of both cortisol and aldosterone (11). These two steroids are produced by aldosterone synthase using 11-deoxycortisol as substrate, although 18OHF can also be produced by 11β-hydroxylase (12, 13). Because aldosterone synthase expression is normally limited to the zona glomerulosa, and 17α-hydroxylase and 11β-hydroxylase necessary for cortisol synthesis occur in the zona fasciculata, production of 18OHF and 18oxoF is normally very low. Their synthesis is dramatically increased in glucocorticoid-remediable aldosteronism (GRA) due to the availability of substrate to the aldosterone synthase expressed in the zona fasciculata in GRA. Levels of 18OHF and 18oxoF were shown to be better than the dexamethasone suppression test for the diagnosis of GRA (14). Elevated hybrid steroid values have also been described in patients with sporadic PA, especially APA, and serum 18OHF concentrations were shown to be higher in PA patients compared with essential hypertensives and normal subjects (15); however, most studies were performed in relatively small numbers of subjects, and therefore the role of hybrid steroid measurement in the diagnosis of PA has not been fully elucidated.

In the present study, we evaluated the role of 18OHB, 18OHF, and 18oxoF in the diagnosis of PA and its subtypes.

Patients and Methods

Patient selection

We studied 143 consecutive hypertensive patients with a positive ARR. Diagnosis of PA was performed as previously described (16). Briefly, the cutoff level considered to be a "positive" ARR was 40 (ng/dl/ng$*$ml^{-1} h^{-1}) (4000 pmol/liter/ng$*$liter^{-1} sec^{-1}) together with an aldosterone level above 15 ng/dl (416 pmol/liter). All antihypertensive drugs were stopped at least 3 wk before the aldosterone and PRA measurements; diuretics and spironolactone were stopped at least 6 and 8 wk before measurements, respectively. Patients who could not remain untreated received an α-blocker (doxazosin) and/or a calcium channel blocker (verapamil or amlodipine) during the screening, until the final diagnosis. Twenty-four-hour urine samples were collected on the day before the confirmatory saline infusion test consisting of an iv saline load (2 liters of 0.9% NaCl infused over 4 h) that was considered positive if posttest aldosterone levels were above 5 ng/dl (138.7 pmol/liter) (17). Patients with PRA below 1 ng ml^{-1}h^{-1}, ARR above 40, aldosterone levels above 15 ng dl^{-1}, and a negative saline load test (SLT), *i.e.* aldosterone levels below 5 ng dl^{-1} after saline infusion, were considered as affected by low-renin essential hypertension (LREH).

Subtype diagnosis was performed by computed tomography (CT) scanning with contrast and fine cuts of the adrenal and subsequent AVS. Sampling was considered successful if the adrenal vein/inferior vena cava cortisol gradient was at least 3 (at least 2 before 2008), and lateralization was defined as an aldosterone/cortisol ratio (A/C) value from one adrenal at least four times the ratio from the other adrenal gland, or three times the A/C of the contralateral with the A/C in the contralateral less than the A/C in the peripheral vein (18). Seventeen of the PA patients had bilaterally normal appearance of the adrenal glands on CT scanning, which in our hands was associated with a very high probability of BAH (95%) (16). These patients were classified as affected by BAH and did not undergo AVS; thus, this diagnosis cannot be considered fully established. However, the statistical analysis was not affected by the inclusion or removal of this group of BAH patients. Finally, all patients with PA were screened for GRA using a long-PCR technique (12). Urinary samples were also collected from previously studied patients with GRA (19), from patients with nonsecreting cortical adrenal

TABLE 1. Hormonal parameters in patients with LREH and PA

	LREH	PA	*P*
n	62	81	
sK^+ (mEq/liter)	4.2 ± 0.4	3.7 ± 0.5	<0.001
PRA (ng $ml^{-1}h^{-1}$)	0.2 (0.1–0.3)	0.2 (0.1–0.3)	n.s.
sAldosterone Up (ng ml^{-1})	20.5 (17.2–26.2)	28.9 (22.1–37.9)	<0.01
ARR	90 (73.1–163.5)	124 (78–221)	n.s.
u18OHF (μg d^{-1})	94 (79–113)	206 (119–338)	<0.001
u18oxoF (μg d^{-1})	2.2 (1.8–2.8)	4.5 (3.2–6.7)	<0.001
sAldosterone Rec	14.3 (9.5–20.8)	26.5 (19–38.2)	<0.001
sAldosterone post-SLT	3 (2–3.5)	11 (7.5–19.5)	<0.001
s18OHB Rec (pg ml^{-1})	567 (462–695)	730 (529–1031)	<0.001
s18OHB post-SLT (pg ml^{-1})	267 (214–313)	417 (303–506)	<0.001
s18OHF Rec (pg ml^{-1})	846 (680–1029)	1225 (960–1770)	<0.001
s18OHF post-SLT (pg ml^{-1})	334 (250–483)	683 (442–1034)	<0.001
s18oxoF Rec (pg ml^{-1})	33 (29–44)	52 (38–97)	<0.001
s18oxoF post-SLT (pg ml^{-1})	11 (7–16)	29 (20–54)	<0.001
s18OHB/aldosterone Rec	45 (32–56)	29 (21–40)	<0.001
s18OHB/aldosterone post-SLT	96 (65–126)	37 (24–49)	<0.001

Data are expressed as mean ± SD or median (25th–75th percentile). K^+, Potassium; Rec, recumbent; Up, upright; s, serum; u, urinary; n.s., not significant. To convert aldosterone to nmol/liter, multiply by 0.0277; to convert PRA to ng $liter^{-1}sec^{-1}$, multiply by 0.2778; to convert 18OHF to μM/d, multiply by 0.00265; to convert 18oxoF to μM/d, multiply by 0.00266; to convert 18OHF from pg/ml to pmol/liter, multiply by 2.64; to convert 18oxoF from pg/ml to pmol/liter, multiply by 2.64; to convert 18OHB from pg/ml to pmol/liter, multiply by 2.76.

tumors (NSAT) incidentally discovered by noninvasive abdominal imaging techniques performed for reasons other than suspected adrenal disease, and from normal subjects. All NSAT subjects received an extensive endocrine evaluation to exclude a functioning adrenal tumor and had adrenal tumors with CT characteristics typical of benign cortical adenomas (20).

The studies were approved by the institutional review board and local ethics committee. All participants gave their written consent.

Hormonal assays

Serum and urinary 18OHF and 18oxoF were measured using a biotin-avidin enzyme-linked immunoassay as previously described (21, 22). Intraassay coefficient of variation was 15% in the lower range and 11% in the higher range. The interassay coefficient of variation was 15% in the lower range and 12% in the higher range. Serum 18OHF assay was performed as described above after an extraction of plasma with a C18 column. 18oxoF was extracted from serum and urine samples on a C2 column as previously described (21, 22). Results were reported per 24-h urinary excretion. The intraassay coefficient of variation was 12% in the lower range and the 8% in higher range. The interassay coefficient of variation was 14% in the lower range and 10% in the higher range. Serum 18OHB was measured by an ELISA method as previously described (23). The intraassay coefficient of variation was 13% in the lower range and 7.5% in the higher range. The interassay coefficient of variation was 14% in the lower range and 9% in the higher range. Biotin-conjugated steroids and anti-18OHB, anti-18OHF, and anti-18oxoF antibodies were produced in the Gomez-Sanchez laboratory. PRA and aldosterone were measured by commercially available kits as described previously (16).

Statistical analysis

Data were analyzed with the Kolmogorov-Smirnov test to determine their distributions. Statistical significance between groups was calculated in normally distributed data by Student *t* test for independent samples and in non-normally distributed data by Kruskal-Wallis and Mann-Whitney *U* test. Bonferroni's corrections were used for multiple comparisons. Data were expressed as median (25th–75th percentiles). Receiver operator characteristic (ROC) analysis was used to determine the test characteristics of the different variables predicting PA diagnosis. ROC curves were compared by the area under the curves. A value of z above the critical level of 1.96 was used to accept the hypothesis that the two areas were different. Correlations were evaluated by Spearman's correlation coefficient.

Results

We studied the hormonal characteristics of 62 patients with essential hypertension (EH) and 81 patients with PA, 20 of which had APA (Table 1). Urinary hormonal measurements were also performed in 24 patients with GRA, 16 patients with NSAT, and 30 normotensive subjects.

Urinary 18OHF and 18oxoF

We observed a significant correlation between urinary levels of 18OHF and 18oxoF ($P < 0.01$) in all subjects (Fig. 1A). After subdividing patients into groups according to their diagnosis we still observed a significant correlation between the two hormones in APA, NSAT, normal subjects, and GRA, but not in BAH and EH patients.

Urinary levels of 18OHF and 18oxoF were significantly higher in GRA patients [median (25th–75th percentiles), 759 (476–1167) and 28 (15–79) μg/d, respectively] compared with all other groups of patients, as expected (Fig. 2, A and B). Patients with PA displayed significantly higher

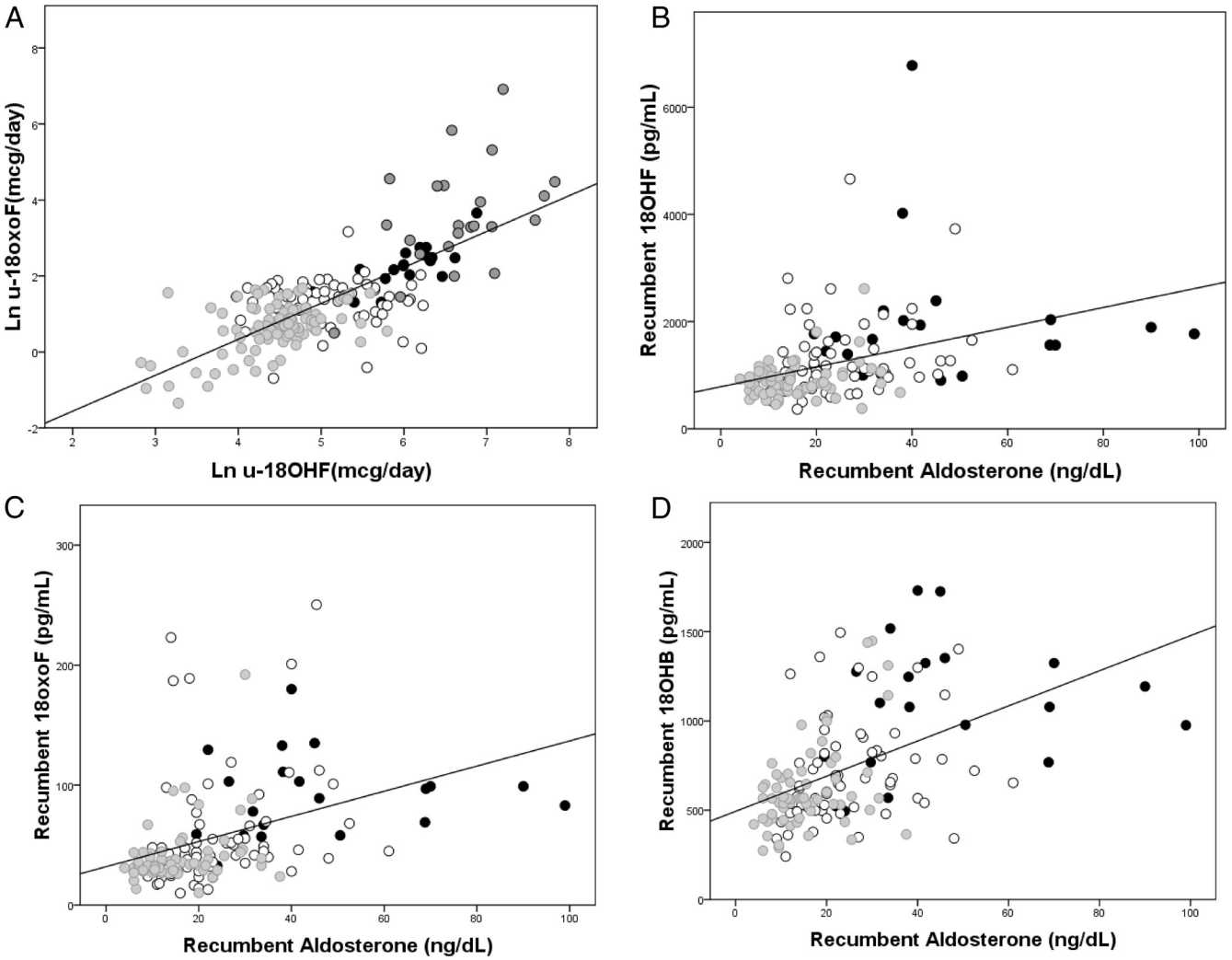

FIG. 1. Associations between aldosterone and hybrid steroids levels in different types of adrenal disorders. A, Urinary 18OHF and 18oxoF levels (log-transformed); B, serum aldosterone and 18OHF; C, serum aldosterone and 18oxoF; D, aldosterone and 18OHB. *Dark gray circles*, GRA patients; *black circles*, APA patients; *white circles*, BAH patients; and *light gray circles*, EH patients, except in panel A, this group also includes normal subjects and NSAT patients.

levels of urinary 18OHF and 18oxoF compared with essential hypertensives (Table 1) and normal subjects [51 (26–77) and 0.8 (0.6–1.1) μg/d, respectively]. In patients with NSAT, urinary 18OHF and 18oxoF levels [111 (99–188) and 2.7 (2.1–4) μg/d, respectively] were significantly higher than in normal subjects and similar to those measured in essential hypertensives and BAH patients (Fig. 2, A and B). Considering PA subgroups, patients with APA displayed 18OHF and 18oxoF levels significantly higher than patients with BAH [407 (290–534) and 9.3 (6.4–12.1) *vs.* 160 (106–258) and 4.3 (2.9–5.4) μg/d, respectively] (Fig. 2, A and B).

Interestingly, only two of 62 (3%) EH patients had 18OHF higher than 190 μg/d, compared with 43 of 81 (53%) PA patients. The highest measured level of 18OHF in EH was 329 μg/d, whereas 21 of 81 (26%) PA patients displayed values greater than 330 μg/d (Fig. 3A). The highest value of 18OHF measured in BAH patients was 507 μg/d, whereas six of 20 (30%) patients with APA displayed values higher than 510 μg/d (Fig. 3A). Furthermore, 13 of 20 (65%) APA patients displayed values higher than 330 μg/d. Finally, the lowest value of 18OHF in APA patients was 133 μg/d, whereas 52 of 63 (83%) EH patients had values lower than 130 μg/d (Fig. 3A). The highest measured value of 18oxoF in EH patients was 5.38 μg/d, whereas 32 of 81 (40%) PA patients and 15 of 20 (75%) APA patients displayed values higher than 5.4 μg/d (Fig. 3B). The lowest urinary 18oxoF value for APA patients was 2.5 μg/d, and 33% of EH patients showed values lower than 2 μg/d (Fig. 3B). In GRA patients, 18OHF and 18oxoF were 10 and 20 times higher than in normal subjects, respectively; of note, despite average 18OHF and 18oxoF levels being significantly higher in GRA than in all other groups, we observed an overlap between levels in APA and GRA patients (Fig. 2, A and B). We also per-

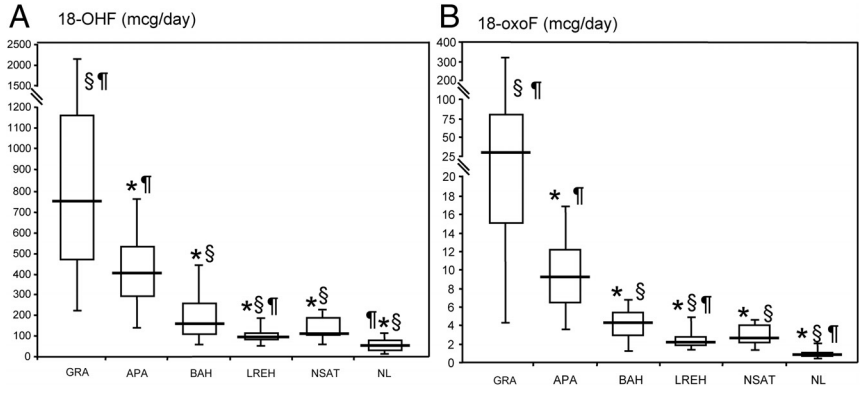

FIG. 2. Steroid levels in different types of adrenal disorders. Levels of 18OHF (A) and 18oxoF (B) in patients with GRA, APA, BAH, LREH, NSAT, and normal subjects (NL). *, $P < 0.001$ compared with GRA; §; $P < 0.001$ compared to APA; ¶, $P < 0.001$ compared with BAH.

formed ROC curves for urinary 18OHF in the diagnosis of PA and observed that it performed better than ARR, with an area under the curve significantly higher (0.82 *vs.* 0.61; $P < 0.05$) (Fig. 3C).

Serum 18OHB, 18OHF, and 18oxoF

We observed a significant correlation between serum aldosterone levels and 18OHB, 18OHF, and 18oxoF levels ($P < 0.01$ for all three comparisons) (Fig. 1, B–D). Furthermore, we also observed a significant correlation between serum 18OHB and 18OHF, between serum 18OHB and 18oxoF, and between serum 18OHF and 18oxoF levels ($P < 0.01$ for all correlations). After subdivision of patients into EH, APA, and BAH groups, correlation between 18OHB and aldosterone, and 18oxoF and aldosterone remained significant in the BAH and EH groups, but not in the APA group, whereas the correlation between 18OHF and aldosterone remained significant only in the BAH group. Furthermore,

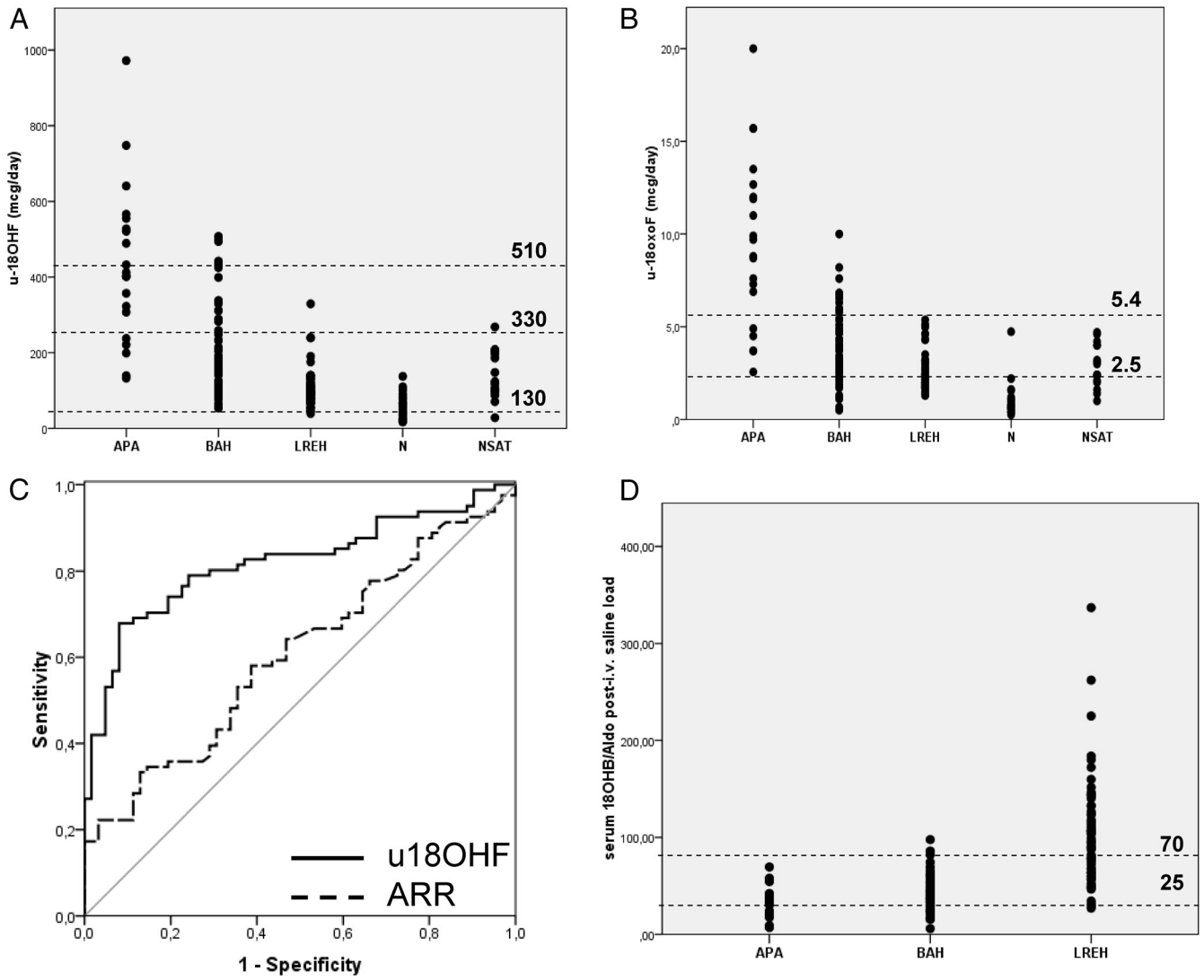

FIG. 3. Distribution of urinary 18OHF levels (A) and urinary 18oxoF levels (B) among different subgroups of patients. ROC curves for urinary 18OHF and ARR (C) in the diagnosis of PA. Distribution of serum 18OHB/aldosterone ratio (D) among different subgroups of patients.

TABLE 2. Hormonal levels in patients with LREH, BAH, and APA

	LREH	BAH	APA
n	62	61	20
sAldo Rec (ng ml^{-1})	14.3 (9.5–20.8)[a]	22 (17–32)[a]	39.1 (31.2–55.1)[a]
sAldo post-SLT (ng ml^{-1})	3 (2–3.5)[a]	9.5 (6.5–13)[a]	25.5 (13.4–47.6)[a]
s18OHB Rec (pg ml^{-1})	567 (462–695)[c]	654 (513–835)[c]	1090 (792–1324)[a]
s18OHB post-SLT (pg ml^{-1})	267 (214–313)[a]	379 (292–484)[a]	625 (465–884)[a]
s18OHF Rec (pg ml^{-1})	846 (680–1029)[a]	1103 (866–1488)[a]	1742 (1432–2023)[a]
s18OHF post-SLT (pg ml^{-1})	334 (250–483)[a]	595 (413–890)[a]	1016 (756–1211)[a]
s18oxoF Rec (pg ml^{-1})	33.1 (29–43.7)[b]	45 (31.6–68)[b]	93 (65–105)[a]
s18oxoF post-SLT (pg ml^{-1})	10.9 (7.1–15.8)[a]	23.1 (13.3–34)[a]	64.5 (37.8–87.3)[a]
s18OHB/Aldo Rec	45 (32–56)	30 (22–42)[a]	27 (18–36)[a]
s18OHB/Aldo post-SLT	96 (65–126)[a]	39 (29–53)[c]	31 (21–41)[c]
Δ>50% sAldo (y/n)	61/1 (98%)[b]	38/24 (61%)[b]	8/12 (40%)[b]
Δ>50% s18OHB (y/n)	44/18 (71%)[b]	25/36 (41%)[b]	3/17 (15%)[b]
Δ>50% s18OHF (y/n)	42/20 (68%)[b]	28/33 (46%)[b]	3/17 (15%)[b]
Δ>50% s18oxoF (y/n)	48/14 (67%)[b]	29/32 (48%)[b]	4/16 (20%)[b]

Data are expressed as median (25th–75th percentiles) or yes/no (percentage). Aldo, Aldosterone; Rec, recumbent; s, serum.

[a] $P < 0.001$; [b] $P < 0.01$; [c] not significant.

correlations between 18OHB, 18OHF, and 18oxoF remained significant in the APA and BAH groups, whereas only the correlation between 18OHF and 18OHB was significant in the EH group.

PA patients displayed higher levels of serum 18OHB compared with EH patients (Table 1); furthermore, APA patients displayed higher levels compared with BAH patients (Table 2). However, we observed a significant overlap between groups; thus, we could not identify cutoff values useful for the distinction among EH, PA, and its subgroups. Furthermore, EH patients displayed significantly higher values of the ratio 18OHB/aldosterone compared with PA patients (Table 1). Of note, APA patients had 18OHB/aldosterone levels not significantly different from BAH patients (Table 2). Once again, the overlap between 18OHB/aldosterone levels among groups did not allow us to identify a useful cutoff for the diagnosis of PA.

Serum 18OHF and 18oxoF were significantly higher in PA patients compared with EH (Table 1); furthermore, APA patients displayed significantly higher levels of 18OHF and 18oxoF compared with BAH (Table 2). Nonetheless, there was significant overlap between groups.

Effects of saline load on steroid levels

Saline infusion significantly reduced 18OHB, 18OHF, and 18oxoF in all groups of patients, although the reduction was not uniform between steroids (Table 2). Aldosterone reduction was greater than 50% in 74% of the patients, compared with 50–57% of the cases for the other three steroids, indicating a greater dependence of aldosterone on the renin-angiotensin system. Even after SLT, we found a significant difference between PA and EH and between APA and BAH for all measured steroids, similar to that observed before infusion (Table 2). Interestingly, the steroid reduction after SLT was much lower for APA patients compared with BAH and EH; in particular, a minority of APA patients displayed a reduction of aldosterone of more than 50% of the basal value (15% for 18OHB and 18OHF and 20% for 18oxoF) compared with around 70% for EH. Unfortunately, due to overlapping values, the reduction of steroid hormones in response to SLT was not sensitive or specific enough to distinguish EH from PA or APA from BAH patients. In most cases (64%), the reduction (more or less than 50%) was discordant between the four hormones. In 10% of cases, none of the hormones were reduced by more than 50%, whereas in 26%, all hormones were reduced by more than 50%. The 18OHB/aldosterone ratio after SLT more than doubled in EH but remained unchanged in PA patients, particularly the APA subgroup (Tables 1 and 2). Of note, the lowest 18OHB/aldosterone ratio after SLT in EH was 27, whereas it was less than 25 in 42% of APA patients (Fig. 3D). Similarly, the highest 18OHB/aldosterone ratio after SLT in APA patients was 69.5, whereas 70% of EH patients displayed values higher than 70 (Fig. 3D).

We also divided APA patients according to the response to posture test (a surrogate of AII response). AII-responsive and AII-unresponsive APA patients were defined as a greater or less than 50% aldosterone increase with upright posture, respectively. Interestingly, hybrid hormone levels tended to be higher in patients with AII-unresponsive APA. Details are available in Supplemental Table 1 (published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Discussion

The Endocrine Society Guidelines recommend that a positive ARR test be followed by a confirmatory test (1) and

PA patients be further studied by CT scanning and AVS. Because LREH accounts for up to 30% (24–26) of the hypertensive population, a high number of patients would undergo a confirmatory test after a positive ARR, and about half of these would undergo CT scanning and AVS to perform subtype diagnosis. It would be useful to identify alternative strategies to reduce the number of confirmatory tests and AVS, which is unavailable in many centers.

In this study, we measured the serum levels of 18OHB and the serum and urinary levels of 18OHF and 18oxoF in hypertensive patients, including 143 with a positive ARR, who were subsequently divided in 62 EH patients and 81 PA patients (comprising 61 BAH and 20 APA patients). We also studied the urinary levels of 18OHF and 18oxoF in 24 GRA patients belonging to two previously described families, 30 normal subjects, and 16 patients with NSAT. We found that urinary 18OHF was the most useful parameter in the differentiation between EH and PA and between APA and BAH. Use of urinary 18OHF levels in patients with a positive ARR could have resulted in the elimination of 36.4% of the iv salt loading confirmatory test and 7.3% of AVS in the present series of PA patients. Therefore, we propose the use of the urinary 18OHF assay in the diagnostic workup for PA. Patients with a positive ARR, but 24-h urinary 18OHF excretion less than 130 μg/d could avoid undergoing confirmatory/exclusion tests because no patient with 18OHF levels below this cutoff had an APA (Fig. 4). Because both LREH patients and PA patients with BAH benefit from MR antagonists, low doses of spironolactone/eplerenone can be added in the treatment of these patients. Patients with a positive ARR and 18OHF excretion between 130 and 510 μg/d could undergo a confirmatory test. However, because patients with a positive ARR and 18OHF excretion greater than 330 μg/d were all PA patients, they could undergo subtype differentiation directly, without performing confirmatory tests, avoiding another 4.9% of confirmatory tests. Finally, patients with 18OHF greater than 510 μg/d all had an APA, and patients with NSAT, which are frequently detected by CT (20), showed 18OHF remarkably lower than this threshold. Therefore, in the presence of a unique solitary nodule after CT scanning, patients with a positive ARR and 18OHF excretion greater than 510 μg/d may undergo unilateral adrenalectomy, especially if young (Fig. 4). Because GRA patients display 18OHF levels higher than 510 μg/d, GRA should always be excluded by long-PCR in all PA patients.

We emphasize that this strategy is still a working hypothesis. In fact, the role of urinary 18OHF in the diagnostic approach to PA must be confirmed in wider populations or in a multicentric study, and the assay must be standardized to be reproducible among centers. In addition, only patients with high or low 18OHF values would avoid further evaluation; patients in the "gray zone" would require other tests for final diagnosis. Further evaluation may show that AVS performance is still superior to 24-h 18OHF excretion for the diagnosis of PA subtypes. However, the limited availability, the high cost, and the lack of standardization of AVS drive the search for alternative or complementary tests.

Urinary 18oxoF levels correlated with those for urinary 18OHF; however, its diagnostic power was slightly lower than urinary 18OHF, and the assay was less reproducible because of lower urinary levels. Therefore, we suggest that the urinary 18oxoF would not add significant information in the diagnosis of PA and its subtypes compared with the urinary 18OHF alone. A recent study demonstrated the role of 18oxoF measured by liquid chromatography-tandem mass spectrometry in samples obtained during AVS from the adrenal veins, in differentiating PA subtypes, further underlying the potential role of hybrid steroids for PA subtype diagnosis (27).

Serum levels of 18OHB, 18OHF, and 18oxoF were higher in PA than in EH patients and in APA compared with BAH patients. However, a significant overlap between groups limits their value in the diagnosis of PA and its subtypes. After saline load, only a minority of APA patients displayed 18OHB, 18OHF, and 18oxoF reductions compared with the basal levels of greater than 50%, whereas aldosterone levels decreased by more than 50% in 40% of

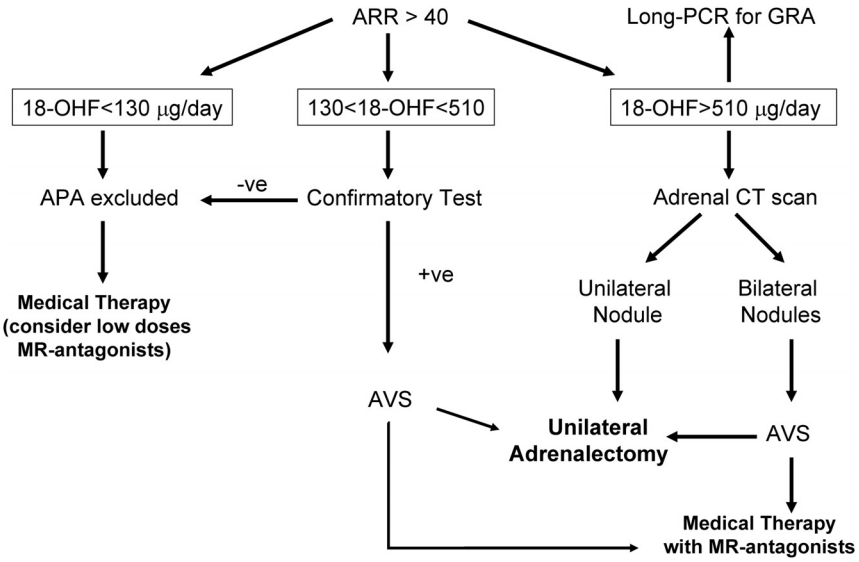

FIG. 4. Suggested flowchart adding urinary 18OHF levels in the diagnosis of PA and its subtypes.

APA patients. Moreover, we observed a significant increase in the 18OHB/aldosterone ratio after saline load in EH, but not in BAH and APA patients. We suggest that the 18OHB/aldosterone ratio may also be useful in the differentiation between EH and PA patients, particularly those with APA. It has been suggested from other studies that 18OHB levels could help differentiate APA from BAH, especially after the posture test (28). We did not measure 18OHB before and after upright posture stimulation; however, basal levels of 18OHB did not help differentiate PA subtypes in the present study.

In conclusion, this is the largest study addressing the role of 18OHF, 18oxoF, and 18OHB in the diagnosis of PA and its subtypes. We demonstrate that none of the steroid assays were sensitive or specific enough to replace a confirmatory test and/or AVS in all patients; however, these more invasive tests may be avoided in a significant number of subjects if the results of the present study are confirmed in a larger multicentric study and the assays become standardized. Meanwhile, AVS is still the only reliable way to distinguish unilateral from bilateral forms of hyperaldosteronism.

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Address all correspondence and requests for reprints to: Paolo Mulatero, Division of Internal Medicine and Hypertension, Azienda Ospedaliero-Universitaria San Giovanni Battista, Via Genova 3, 10126 Torino, Italy. E-mail: paolo.mulatero@libero.it.

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