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Clinical Research Article

Personalized Noninvasive Diagnostic Algorithms Based on Urinary Free Cortisol in ACTH-dependant Cushing's Syndrome

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Abstract

Context: Current guidelines for distinguishing Cushing's disease (CD) from ectopic ACTH secretion (EAS) are questionable, as they use pituitary magnetic resonance imaging (MRI) as first-line investigation for all patients. CRH testing is no longer available, and they suggest performing inferior petrosal sinus sampling (BIPPS), an invasive and rarely available investigation, in many patients.

Objective: To establish noninvasive personalized diagnostic strategies based on the probability of EAS estimated from simple baseline parameters.

Design: Retrospective study.

Setting: University hospitals.

Patients: Two hundred forty-seven CD and 36 EAS patients evaluated between 2001 and 2023 in 2 French hospitals. A single-center cohort of 105 Belgian patients served as external validation.

Results: Twenty-four-hour urinary free cortisol (UFC) had the highest area under the receiver operating characteristic curve for discrimination of CD from EAS (.96 [95% confidence interval (CI), .92-.99] in the primary study and .99 [95% CI, .98-1.00] in the validation cohort). The addition of clinical, imaging, and biochemical parameters did not improve EAS prediction over UFC alone, with only BIPPS showing a modest improvement (C-statistic index .99 [95% CI, .97-1.00]). Three groups were defined based on baseline UFC: < 3 (group 1), 3-10 (group 2), and $> 10 \times$ the upper limit of normal (group 3), and they were associated with 0%, 6.1%, and 66.7% prevalence of EAS, respectively. Diagnostic approaches performed in our cohort support the use of pituitary MRI alone in group 1, MRI first followed by neck-to-pelvis computed tomography scan (npCT) when negative in group 2, and npCT first followed by pituitary MRI when negative in group 3. When not combined with the CRH test, the desmopressin test has limited diagnostic value.

Conclusion: UFC accurately predicts EAS and can serve to define personalized and noninvasive diagnostic algorithms.

Key Words: Cushing's disease, ectopic ACTH syndrome, diagnostic algorithm, inferior petrosal sinus sampling, desmopressin test

Significance Statement

Recent guidelines for the differentiation between CD and EAS recommend first performing pituitary MRI in all patients. BIPSS or a noninvasive strategy combining the CRH and desmopressin tests in association with a neck-to-pelvis CT scan can be performed when no pituitary adenoma is visible. However, prioritizing pituitary MRI as a first-line investigation may be of little use in patients with highly probable EAS, and the CRH test is no longer available. We show in the one of largest cohort published to date that the increase in UFC has excellent performance in discriminating between CD and EAS. Based on this estimated prevalence, we determined personalized diagnostic algorithms avoiding the CRH test and reducing the need for BIPSS.

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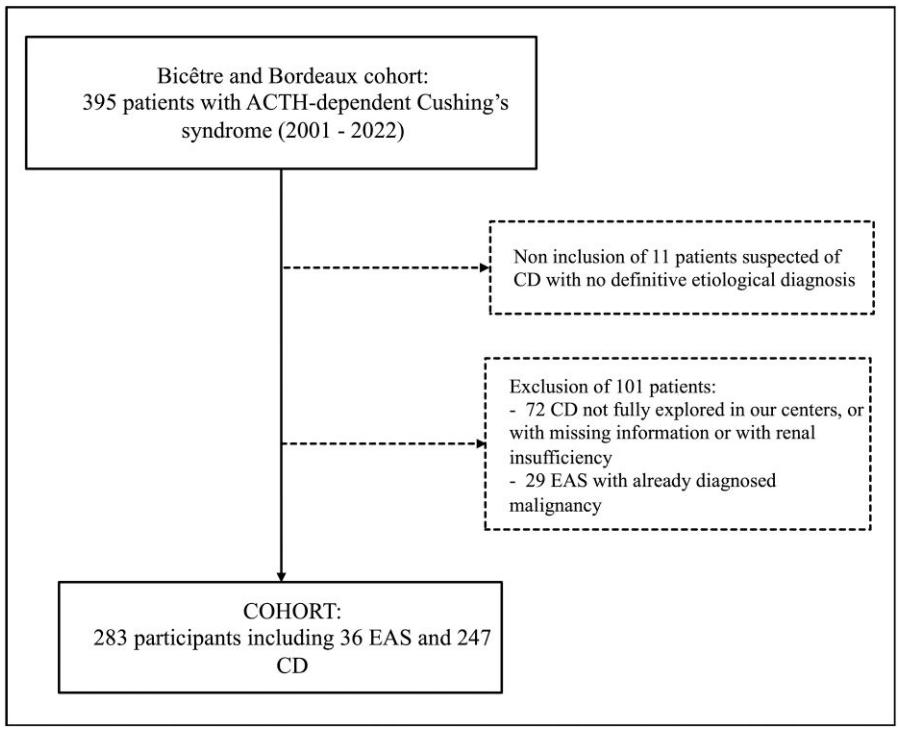

Figure 1. Flow chart of the study.

Abbreviations: CD, Cushing's disease; EAS, ectopic ACTH syndrome.

The differential diagnosis between Cushing's disease (CD) and ectopic ACTH syndrome (EAS) is often challenging. A large international group of experts from the Pituitary Society recently proposed a diagnostic algorithm involving a pituitary magnetic resonance imaging (MRI) scan first in all patients with ACTH-dependent Cushing's syndrome (CS) given the predominant prevalence of CD (1). When no obvious pituitary adenoma is seen, one option is to perform bilateral petrosal sinus sampling (BIPSS), which is considered the most effective diagnostic procedure. A noninvasive alternative aimed at reducing the indication for BIPSS is a combination of dynamic CRH and desmopressin tests in association with a neck-to-pelvis computed tomography scan (npCT), as suggested by the results of our previous study (1, 2).

However, EAS is often an endocrine emergency associated with intense hypercortisolism responsible for numerous comorbidities (3, 4). Prioritizing pituitary MRI as a first-line investigation may be of little use in patients with highly probable EAS or may even be harmful given the urgency of treating intense hypercortisolism that is frequently associated and the delay commonly observed in obtaining a high-quality pituitary MRI. Similarly, dynamic endocrine tests are time-consuming and often provide delayed results. Alternatively, performing an invasive procedure like BIPSS is questionable in a fragile patient with EAS with associated hypercoagulability (4).

Our study aimed to establish personalized diagnostic strategies based on the hypothetical prevalence of EAS. Simple parameters, such as age, sex, levels of urinary free cortisol (UFC), plasma ACTH concentrations, and the presence of hypokalemia, are associated with EAS and may serve to distinguish EAS from CD (3-5). Consequently, we first retrospectively evaluated, in a large cohort of patients, the discriminative values of simple clinical and biochemical parameters to define groups at varying risk of EAS. We then analyzed the real-life performance of complementary investigations in our cohort in order to propose diagnostic algorithms according to EAS prevalence while considering the lack of availability of the CRH test with the aim of reducing the need for BIPSS in a selected subset of patients. In addition, we examined the discriminatory performance of simple baseline biochemical parameters in an independent cohort of patients with ACTH-dependent CS.

Patients and Methods

Patients

The data from 283 patients with ACTH-dependent CS, who were referred between 2001 and 2023 to 2 French university hospitals (Centre Hospitalier Universitaire of Bordeaux and Bicêtre) were retrospectively analyzed. The data were collected under conditions of regular clinical care. All patients were informed of the secondary use of their healthcare data for research purposes in research projects and gave their consent. The study protocol was approved by the local ethics committees of both Bordeaux and Bicêtre hospitals.

Both centers have extensive experience in the management of CS and follow similar investigational and therapeutic protocols. The data of 394 patients were examined, and 283 were included in the study (Fig. 1). The diagnosis of CD was based on histopathology, biochemical remission following transsphenoidal surgery, or a central-to-peripheral ACTH-gradient on BIPSS. The diagnosis of EAS was based on histopathology or the absence of ACTH gradient on BIPSS. Patients with diagnosed cancers prior to referral or who had evident metastatic tumors at presentation were excluded from the EAS group. Part of this cohort has already been the subject of a published study (2). The present series increases this by adding 89 patients (80 CD and 9 EAS) who meet our inclusion criteria. For external validation, we used data from a Belgian cohort of 90 CD patients and 15 EAS

patients who were referred to the University Clinic of Saint-Luc, Brussels, between 2000 and 2022, using similar inclusion criteria.

Methods

Patients with ACTH-dependent CS first underwent CRH and desmopressin tests and pituitary MRI (2). Patients without an established etiology then underwent a npCT. BIPSS was performed if required only after this first set of investigations. Some CD patients underwent npCT while waiting for the appointment for pituitary MRI, which turned out to be positive. Pituitary surgery was performed by expert neurosurgeons when CD was suspected even when MRI was negative. When EAS was suspected, complementary imaging investigations such as Octreoscan, DOTA-TOC positron emission tomography (PET), thoraco-abdominal MRI, 18FDG PET-CT, and 18 F-Dopa PET-CT were performed (2).

Endocrine Tests and Assays

Hypokalemia was defined as a categorical variable in patients with a serum potassium < 3.5 mmol/L. Patients who were receiving potassium supplementation or spironolactone were excluded from all analyses including kalemia (n = 10). UFC was the mean of 2 or 3 24-hour urine baseline collections in the vast majority of patients. UFC is expressed relative to the upper limit of normal (ULN) range of each assay. In accordance with our objectives and due to the shortage of CRH in 2022, the results of CRH tests were not analyzed. The desmopressin test was analyzed as previously described (2): a relative increase in serum cortisol > 18% associated with a relative increase in plasma ACTH > 33% was in favor of CD. BIPSS was performed coupled with CRH or desmopressin injection after ensuring that the patients were in a state of active hypercortisolism (2). Criteria consistent with CD were a baseline central/peripheral ACTH ratio ≥ 2.0 or a poststimulation ratio ≥ 3.0. Hormone assays are described in the supplementary methods (6).

Imaging Studies

Imaging studies were analyzed and discussed in multidisciplinary team meetings involving expert endocrinologists, radiologists, and neurosurgeons who knew the results of biochemical investigations and the level of suspicion of EAS. The diagnosis of pituitary adenoma was based on qualitative analysis of MRI scans without using the 6 to 9 mm thresholds of current guidelines (1). However, only lesions > 3 mm were considered as positive while smaller and equivocal pituitary MRI images were considered negative. Pituitary MRI and npCT methodology is described in the supplementary methods (6).

Statistical Analysis

Categorical variables are presented as the number of participants with corresponding percentage. Continuous variables are expressed as mean ± SD or as median (interquartile range [IQR]) for those with skewed distribution. Chi-squared, ANOVA, or Kruskal–Wallis/Wilcoxon rank sum tests were used to compare baseline characteristics of participants with EAS vs those with CD. Spearman's test was used to assess correlations between hormonal parameters.

We computed sensitivity, specificity, positive negative predictive values, and negative predictive values with associated 95% confidence interval (CI) of UFC, midnight serum cortisol, and plasma ACTH to identify individuals with EAS vs those with CD. Receiver operating characteristic analyses were used to plot areas under the curve (AUC) of these hormonal parameters. UFC that had the highest AUC was used as a reference model to test the prognostic performance of each clinical, hormonal, or imaging parameter to identify participants with EAS. We then calculated the C-statistics index to compare the ability to predict EAS using model that included only UFC vs models that included UFC plus different clinical, hormonal, or imaging parameters. CIs were generated from 1000 times parametric bootstrapping. Model calibration was checked using the Hosmer and Lemeshow test.

We also performed 2 sensitivity analyses to compute the AUC of the UFC × ACTH product and the AUC of UFC assessed exclusively by immunoassays for EAS discrimination.

Statistical analyses were performed by using Stata software version 15 (StataCorp; www.stata.com) and JMP software version 14 (SAS Institute; www.jmp.com). Two-sided *P*-values < .05 were considered significant.

Results

Characteristics of Patients and Result of Investigations

Two hundred forty-seven CD patients (205 females and 42 males) aged 41.2 ± 14.2 years and 36 EAS patients (22 females and 14 males) aged 43.5 ± 19.0 years were included in the study (Table 1). The diagnosis of CD was established in 213 patients based on histopathological analysis or on remission of CD following transsphenoidal surgery. The CD diagnosis was based on the results of BIPSS in 34 patients. EAS was histologically confirmed in 33 patients and diagnosed on the results of BIPSS in 3 patients with occult tumors [Supplementary Table S1 (6)].

Plasma concentrations of different hormonal parameters are shown in Table 1 and their correlations in the Supplementary Table S2 (6). The highest correlation was observed between UFC and midnight plasma cortisol and the lowest between ACTH and kalemia.

Patients with EAS, compared with those with CD, were more frequently men, while mean ages were comparable. They had higher values of UFC, midnight serum cortisol, and 8 AM plasma ACTH. Median UFC was 10 times higher in EAS patients than in CD patients. Twenty CD patients (8%) had UFC within normal values, while the lowest value among EAS patients was 3.2 ULN. Hypokalemia and negative responses to the desmopressin test were more frequently observed in EAS than in CD patients.

In EAS patients, CT identified 24 (66.7%) tumors at presentation. One 15 mm false-positive thymic cyst was observed in 155 CD patients. Pituitary MRI identified 118 microadenomas and 49 macroadenomas in the 247 CD patients. The median size of microadenomas on MRIs was 7 mm (IQR 5, 10) and 50% were < 6 mm in size. One 4 mm false-positive pituitary image was observed in the 36 EAS patients. BIPSS was associated with 3 false negatives in 98 CD patients and 1 false positive in 17 EAS patients. The diagnostic performance of the investigations is shown in Table 2.

Diagnostic Performance of Basic Biochemical Investigations

The cutoffs of 10 × ULN, 750 nmol/L, and 100 pg/mL for UFC, midnight cortisol, and ACTH provided the

Table 1. Characteristics of patients

	n	Cushing's disease	EAS	*P*-value
n (%)		247 (87.3)	36 (12.7)	
Age (years)	283	41.2 ± 14.2	43.5 ± 19.0	.40
Female (%)	283	205 (83)	22 (61)	.006
UFC (× ULN)	283	2.9 (1.7, 4.8)	29.7 (13.4, 57.2)	<.0001
Midnight plasma cortisol (nmol/L)	279	435 (349, 553)	907 (670, 1490)	<.0001
Plasma ACTH (pg/mL)	283	59 (40, 86)	118 (87, 176)	<.0001
Hypokalemia	257	35 (15.6)	27 (84.4)	<.0001
Positive desmopressin test	277	201 (83.1)	7 (20.6)	<.0001
Abnormal pituitary MRI	283	167 (67.6)	1 (2.9)	<.0001
Abnormal neck-to-pelvis CT scan	191	1 (.6)	24 (66.7)	<.0001
ACTH gradient at BIPSS	116	96 (97)	1 (6)	<.0001

Categorical data expressed as numbers (%) and continuous variables as mean ± SD or median (interquartile range) for those with skewed distribution.
Abbreviations: BIPSS, bilateral inferior petrosal sinus sampling; CT, computed tomography; EAS, ectopic ACTH syndrome; MRI, magnetic resonance imaging; UFC, urinary free cortisol; ULN, upper limit of normal.

Table 2. Diagnostic performance of the desmopressin test, pituitary MRI, BIPSS, and neck-to-pelvis CT scan for the diagnosis of Cushing's disease and ectopic ACTH syndrome

	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Diagnosis of Cushing's disease				
Pituitary MRI	.67 (.62-.73)	.97 (.92-1.00)	.99 (.98-1.00)	.30 (.22-.39)
Diagnosis of ectopic ACTH syndrome				
Neck-to-pelvis CT scan	.67 (.51-.82)	.99 (.98-1.00)	.96 (.88-1.00)	.93 (.89-.97)
BIPSS	.94 (.83-1.00)	.97 (.93-1.00)	.85 (.69-1.00)	.99 (.97-1.00)
Desmopressin test	.80 (.67-.93)	.83 (.78-.88)	.41 (.29-.52)	.97 (.94-.99)

Abbreviations: BIPSS, bilateral inferior petrosal sinus sampling; CI, confidence interval; CT, computed tomography; MRI, magnetic resonance imaging; NPV, negative predictive value; PPV, positive predictive value.

Table 3. Diagnostic performance of basic biochemical investigations for the diagnosis of ectopic ACTH syndrome

Cut-off (nmol/L)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Youden index (95% CI)
UFC (×ULN)					
≥3	1.00 (1.00-1.00)	.51 (.45-.57)	.22 (.16-.29)	1.00 (1.00-1.00)	.51 (.45-.57)
≥10	.80 (.67-.93)	.94 (.91-.97)	.67 (.53-.81)	.97 (.95-.99)	.74 (.58-.90)
≥20	.55 (.39-.72)	.99 (.97-1.00)	.87 (.73-1.00)	.94 (.91-.97)	.54 (.36-.72)
Midnight plasma cortisol (nmol/L)					
≥500	.94 (.86-1.00)	.63 (.57-.69)	.27 (.19-.34)	.99 (.97-1.00)	.57 (.43-.69)
≥750	.71 (.56-.86)	.94 (.91-.97)	.62 (.48-.77)	.96 (.93-.98)	.65 (.47-.83)
≥1000	.40 (.24-.56)	.99 (.97-1.00)	.82 (.64-1.00)	.92 (.89-.95)	.39 (.21-.56)
8 AM plasma ACTH (pg/mL)					
≥50	.91 (.82-1.00)	.38 (.32-.44)	.18 (.12-.23)	.97 (.93-1.00)	.29 (.14-.44)
≥100	.69 (.54-.84)	.83 (.79-.88)	.38 (.26-.50)	.95 (.92-.98)	.52 (.33-.72)
≥200	.17 (.05-.29)	.98 (.96-.99)	.50 (.22-.78)	.89 (.86-.93)	.15 (.01-.28)

Abbreviations: CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value; UFC, urinary free cortisol; ULN, upper limit of normal.

best combination of specificity and specificity with the highest Youden index to identify patients with EAS (Table 3).

The AUC of UFC was .96 (95% CI, .93-.99) while those for midnight serum cortisol and plasma ACTH were .91 (95% CI, .86-.96) and .82 (95% CI, .75-.90), respectively (Fig. 2A). The AUC of UFC was similar when we considered only patients (n = 199) with UFCs assessed by immunoassays (.97, 95% CI, .94-.99).

The addition of clinical (age, sex), imaging (pituitary MRI, CT scan), and biochemical parameters (ACTH, desmopressin test) did not provide any significant improvement in

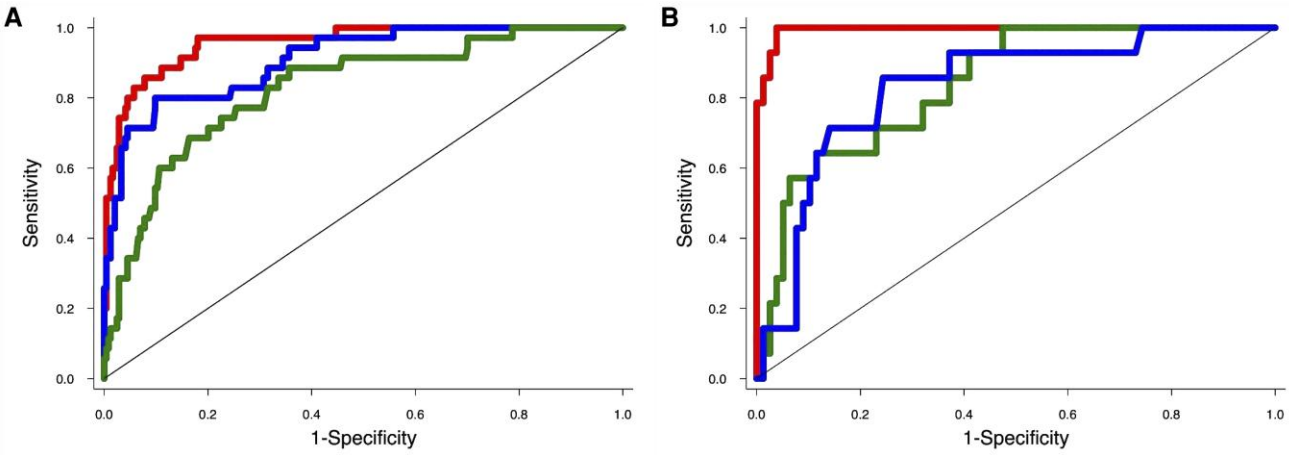

Figure 2. (A and B). Performance of midnight plasma cortisol (blue line), UFC (red line), and plasma ACTH (green line) to discriminate ectopic ACTH syndrome using receiver operator characteristic curves. Results obtained from the main cohort (A) and from the external validation cohort (B).
Abbreviations: UFC, urinary free cortisol.

Table 4. Discrimination of ectopic ACTH syndrome according to clinical, biochemical, and imaging parameters

Models	C-statistic index (95% CI)	*P*
UFC alone	.96 (.92-.99)	
UFC plus		
Age	.96 (.92-.99)	.71
Age and sex	.94 (.90-.98)	.22
Age, sex, and hypokalemia[a]	.96 (.91-1.00)	.39
Age, sex, and plasma ACTH	.95 (.90-1.00)	.88
Age, sex, and MRI	.95 (.91-.99)	.99
Age, sex, and neck-to-pelvis CT scan[a]	.95 (.90-.99)	.84
Age, sex, MRI, and neck-to-pelvis CT scan[a]	.94 (.90-.99)	.69
Age, sex, MRI, and desmopressin test	.95 (.92-.99)	.92
Age, sex, MRI, desmopressin test, and neck-to-pelvis CT scan	.96 (.92-.99)	.82
Age, sex, and BIPSS[a]	.99 (.98-1.00)	.02

C-statistics index computed to compare the ability to predict EAS using the model that included UFC plus various clinical, biochemical, and imaging parameters compared to the model that included UFC alone.
Abbreviations: BIPSS, bilateral inferior petrosal sinus sampling; CI, confidence interval; CT, computed tomography; MRI, magnetic resonance imaging; UFC, urinary free cortisol.
[a]Analyses performed in participants for whom data were available as shown in Table 1.

C-statistic index compared to UFC alone to identify individuals with EAS (Table 4). Only BIPSS provided a significant enhancement in C-statistic index when added to UFC (C-index .99, 95% CI, .98-1.00, $P = .02$).

Flow Diagram of Diagnostic Approaches According to UFC Values

Three groups of patients were considered for the prevalence of EAS based on UFC levels (Fig. 3A).

Group 1 (n = 126) consisted of patients with a baseline UFC < 3 × ULN [Supplementary Fig. S1 (6)]. This group included only CD patients (44.5% of the cohort and 50.4% of CD patients). Consequently, the 87.3% pretest probability of CD in the whole cohort increased to 100% when UFC was < 3 × ULN. Pituitary MRI identified an adenoma in 66.7% of patients. False negatives for the desmopressin test were observed in 12.2% and 11.3% of CD patients with positive and negative MRI, respectively. npCT was performed in 41 of the 42 patients with a negative pituitary MRI and was always normal. Two false-negative results of BIPSS were observed in the 36 CD patients with negative MRI.

Group 2 (n = 114) included patients with a baseline UFC ranging between 3 × ULN and 10 × ULN [Supplementary Fig. S2 (6)]; 40.3% of patients in the cohort were in this group, with CD and EAS representing 93.8% and 6.2% of patients, respectively. Age, sex ratio, and ACTH concentrations were similar between the CD and EAS patients [Supplementary Table S3 (6)]. MRI identified a pituitary adenoma in 68.2% of CD patients with no false positive among the 7 EAS patients. The desmopressin test was negative in 17/72 (23.6%) CD patients who had a positive MRI. Consequently, the combination of MRI + desmopressin decreased the sensitivity of MRI alone from 68.2% to 52.4% without any gain in specificity. Due to a negative desmopressin test, 8 of 17 CD patients with positive MRI underwent unnecessary npCT and BIPSS.

CD and EAS represented 85.0% and 17.1% of the 41 patients with a negative pituitary MRI, respectively. In this subgroup, the desmopressin test was positive in 29/33 of CD patients and in 3 of 7 EAS patients. npCT was negative in CD patients of this subgroup and identified 5 of 7 EAS. BIPSS had 1 false negative in the 31 CD patients and no false positives in the 4 EAS patients.

Group 3 (n = 43) consisted of patients with a baseline UFC > 10 × ULN [Supplementary Fig. S3 (6)]; 15.2% of participants were in this group. The prevalence of EAS increased from 12.7% in the entire cohort to 67.4% in this group, while that of CD decreased from 87.3% to 32.6%. 5.7% of CD patients vs 80.6% EAS patients had a UFC > 10 × ULN, respectively. False negatives and positives in the desmopressin test were observed in 4 of 13 CD patients and 4 of 27 EAS patients, respectively. npCT identified 65.5% of EAS at presentation with no false positives. The prevalence of EAS and CD was 41.7% and 58.3%, respectively, in the remaining 24 patients with no npCT or negative npCT. In

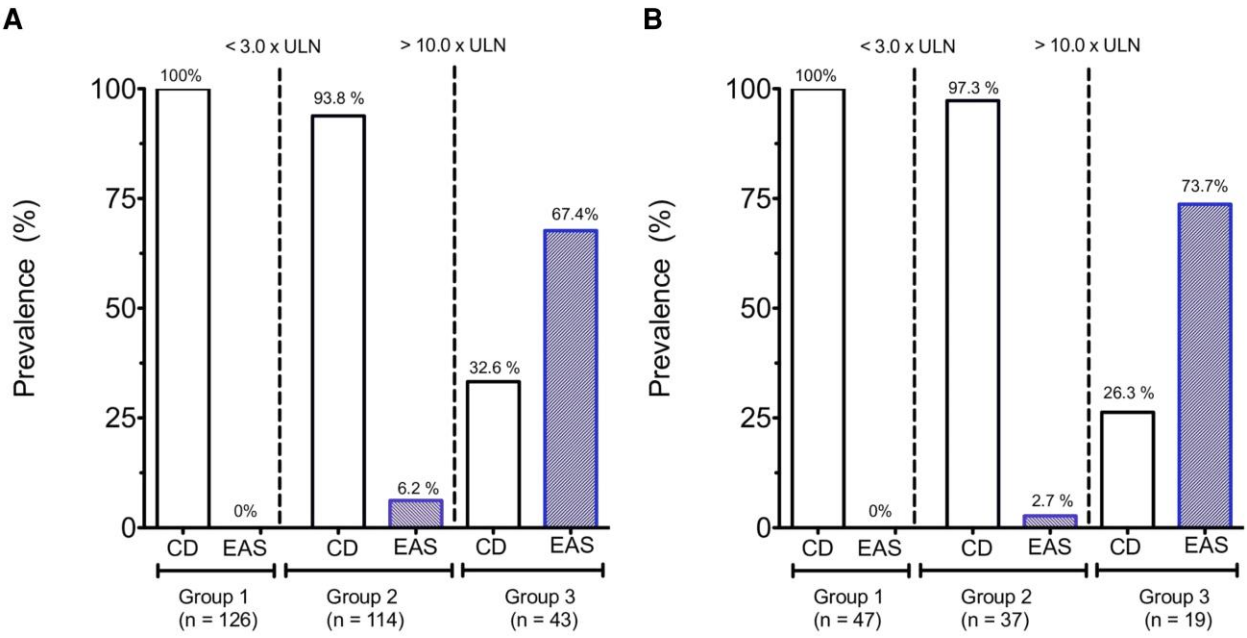

Figure 3. (A and B). Prevalence of CD (white columns) and ectopic ACTH syndrome (blue columns) according to baseline urinary free cortisol. Results obtained from the main cohort (A) and from the external validation cohort (B). N indicates the number of patients in the UFC subgroups.

Abbreviations: CD, Cushing's disease; EAS, ectopic ACTH syndrome; ULN, upper limit of normal.

these patients, MRI identified a pituitary adenoma in 10/14 CD patients and was associated with 1 false positive in an EAS patient. BIPSS gave no false negatives in 7 CD patients and 1 false positive in 13 EAS patients.

Results of the External Validation Cohort

The external cohort included 71 (79%) women and 19 (21%) men, aged 42.2 ± 14.6 years with CD and 11 men and 4 women aged 49.1 ± 15.4 years with EAS [Supplementary Table S3 (6)]. The median UFC was 2.9 × ULN (IQR 1.9, 5.7) in CD patients and 22.2 × ULN (IQR 18.4, 83.5) in EAS patients. The lowest UFC in EAS patients was 9.69 × ULN. The AUC for UFC, midnight cortisol, and 8 AM plasma ACTH were .99 (95% CI, .98–1.00), .84 (95% CI, .74–.94), and .83 (95% CI, .72-.94), respectively (Fig. 2B).

As shown in Fig. 3B, 49 patients (46.7%) had UFC < 3 × ULN and all of these had CD. Thirty-seven patients (35.2%) had UFC between 3 and 10 × ULN. The prevalence of CD and EAS in this group was 97.3% and 2.7%, respectively. Nineteen patients (18.1%) had UFC > 10 × ULN. The prevalence of CD and EAS in this group was 26.3% and 73.7%, respectively (Fig. 3B). npCT was positive in 9 of the 15 EAS patients at presentation.

Discussion

The main finding from of our study is that UFC, both in our large cohort and in an independent validation cohort, shows excellent performance in discriminating between CD and EAS. Grouping patients by UFC levels identified 3 groups with zero, very low, or high prevalence of EAS. Thanks to this accurate estimation of EAS prevalence, distinct diagnostic algorithms can be proposed that allow rapid, efficient, and economical use of complementary investigations. In a noninvasive perspective aimed to reduce the use of BIPSS, neck-to-pelvis CT and pituitary MRI are useful in diagnostic algorithms after first categorizing by UFC level. When not associated with the CRH test, the desmopressin test has only limited indication in the diagnostic algorithm.

In the primary and the validation cohorts, UFC levels < 3 × ULN excluded diagnosis of EAS. Exceptional cases of EAS with a UFC < 3 × ULN have been published but often with details missing (4, 7-10). A number of these came from oncology departments, and the intensity of cortisol secretion was very variable, or Cushing's syndrome appeared after an initial oncologic presentation. These situations differ significantly from the presentation of EAS with clinical CS and no history of cancer in "endocrinological" recruitment as observed in our study. We therefore suggest first performing pituitary MRI in this subgroup (corresponding to 45% of patients) to provide anatomical information for the neurosurgeon, with no additional investigation necessary if MRI is positive. In patients with negative MRI (33% of this group), subsequent investigation is debatable. Several studies claim that the results of pituitary surgery are similar when pituitary MRI is either normal or shows a microadenoma and that an expert surgeon has "better eyes" than the MRI (11, 12). Proponents of this view might therefore consider performing pituitary surgery without further investigation. npCT was not associated with any false positive in this group and was associated with fewer false positives than the desmopressin test in the entire cohort (Table 1). Therefore, for surgeons who need increased confidence in the diagnosis of CD, and from a noninvasive perspective, one could consider performing npCT in patients with a negative pituitary MRI in order to exclude an exceptional case of mild EAS (Fig. 4).

The prevalence of EAS was very low in patients with a UFC between 3 and 10 × ULN (6.1% and 2.7% in the main and validation cohorts, respectively). Given the

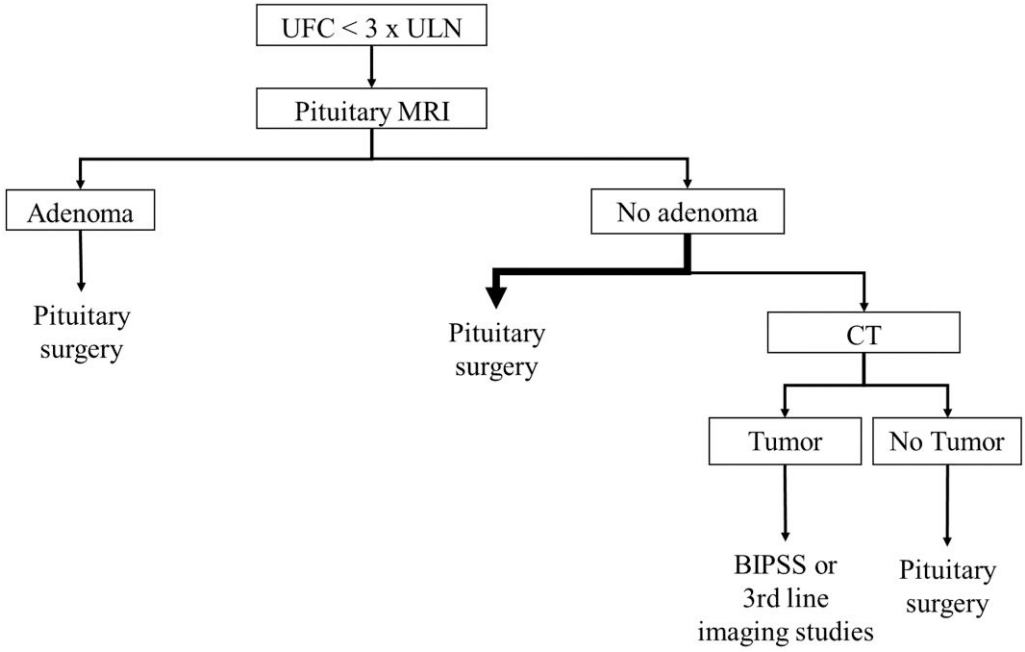

Figure 4. Diagnosis algorithm for group 1 patients (UFC < 3 × ULN). Negative MRI after multidisciplinary examination includes no visible adenoma, images ≤ 3 mm in size, and equivocal images.

Abbreviations: MRI, magnetic resonance imaging; UFC, urinary free cortisol; ULN, upper limit of normal.

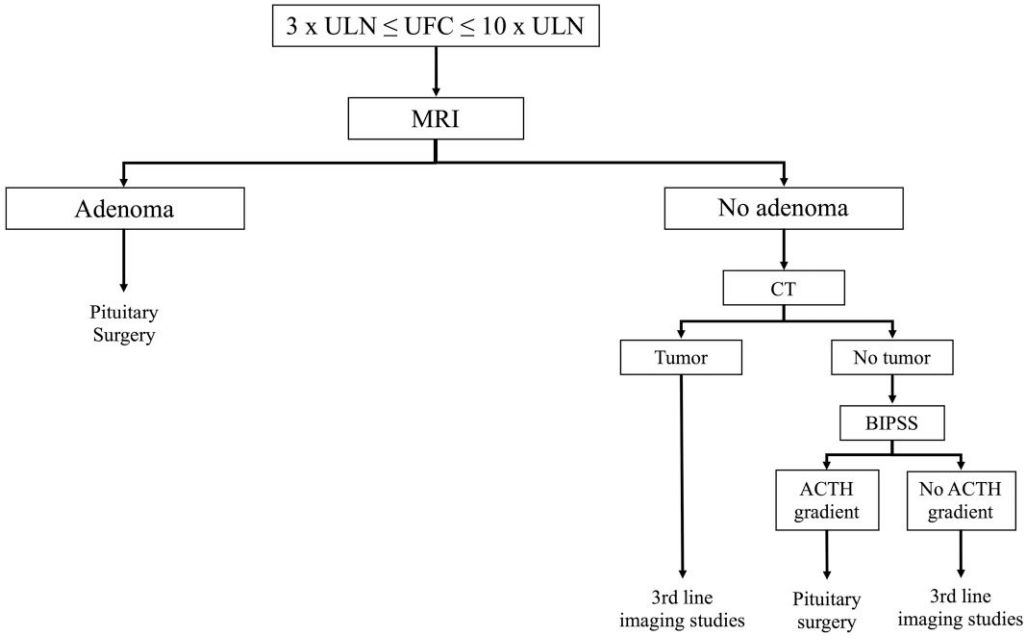

Figure 5. Diagnosis algorithm for group 2 patients (3 × ULN ≤ UFC ≤ 10 × ULN). Negative MRI after multidisciplinary examination includes no visible adenoma, images ≤ 3 mm in size and equivocal images.

Abbreviations: BIPSS, bilateral inferior petrosal sinus sampling; CT, neck-to-pelvis computed tomography scan; MRI, magnetic resonance imaging; UFC, urinary free cortisol; ULN, upper limit of normal.

higher prevalence of CD, we favor first performing pituitary MRI. Coupling pituitary MRI to desmopressin testing decreased the diagnostic performance of MRI, resulting in unnecessary npCT and BIPSS. Similarly, the desmopressin test had suboptimal performance in patients with negative pituitary MRI, while npCT identified 5 of 7 ectopic tumors without a single false positive. We therefore recommend performing npCT when pituitary MRI is negative and reserving BIPSS only for npCT-negative cases (Fig. 5).

Considering the high prevalence of EAS (67.4%) in patients with a UFC > 10 × ULN, the wide availability of prompt npCT, and its 65% sensitivity, we suggest performing a npCT first in this UFC subgroup. A roughly similar prevalence of EAS (74%) and sensitivity of npCT (60%) were observed in the validation cohort. No additional biochemical investigation

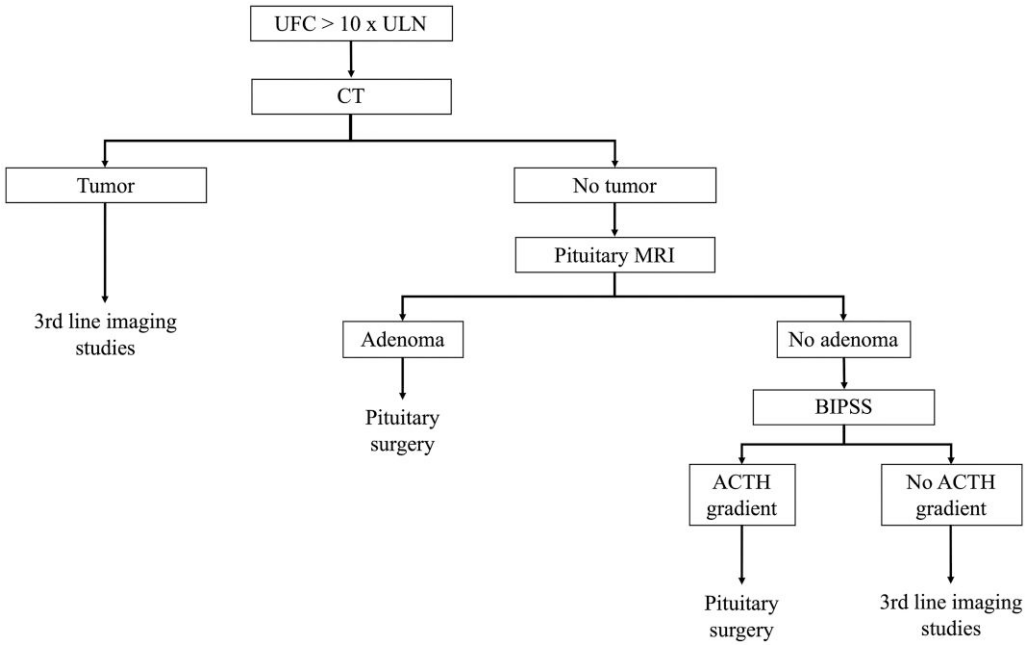

Figure 6. Diagnosis algorithm for group 3 patients (UFC > 10 × ULN). Negative MRI after multidisciplinary examination includes no visible adenoma, images ≤ 3 mm in size, and equivocal images.

Abbreviations: BIPSS, bilateral inferior petrosal sinus sampling; CT, neck-to-pelvis computed tomography scan; MRI, magnetic resonance imaging; UFC, urinary free cortisol; ULN, upper limit of normal.

would be necessary when npCT is positive, which represents almost two-thirds of this group. Nevertheless, according to expert recommendations, a positive npCT should lead to confirmatory functional imaging studies such as DOTATOC-PET or FDG-PET (1, 3, 4, 10).

The prevalence of CD was roughly similar to that of EAS in patients with a UFC > 10 × ULN and a negative npCT. We therefore suggest further diagnostic investigation with a pituitary MRI, which identified pituitary adenomas with a similar sensitivity to that observed in the entire cohort (Fig. 6). As in some published series, only 1 false positive on MRI was observed in an EAS patient (9). The rarity of this occurrence may be explained by the expertise of our multidisciplinary teams who were aware of the degree of suspicion of EAS and the decision to treat equivocal images as negatives (13, 14). However, the high prevalence of EAS favors the occurrence of false positives, and, therefore, particular caution in analyzing pituitary MRI is recommended in this UFC group. Again, in this case, the desmopressin test cannot be recommended to aid interpretation of equivocal MRI images given its suboptimal performance. The small number of patients in this UFC group with both negative pituitary MRI and CT prevents strong recommendations being made for further investigations. Functional imaging as the next investigation may be an option to avoid BIPSS since, in a recently published meta-analysis, DOTATOC-PET was found to be positive in 50% of patients with occult EAS (15).

Based on these algorithms, BIPSS could have been performed in only 34/114 patients of group 2 and in 13/43 patients of group 3, ie, 17% of the whole cohort.

Overall, our study suggests that the desmopressin test is of limited value in a diagnostic algorithm even if its diagnostic performance was only slightly lower than that found in our previous study (2). Clearly, the predictive value of the test considered alone is significantly lower than that of the CRH + desmopressin tests in tandem (2, 16, 17). Importantly, several groups have reported that the disappearance of an aberrant preoperative response to desmopressin following pituitary surgery in CD patients helps to establish the probability of postoperative long-term remission (18, 19). Therefore, the desmopressin test could be performed for postoperative purposes prior to pituitary surgery in CD patients.

Our study has several limitations. EAS is associated with a shorter duration of clinical symptoms (3, 4), a parameter that we were unable to accurately analyze retrospectively from medical charts. Cyclic cases of EAS have also been reported (20) and constitute a major trap, emphasizing the importance of repeating UFC measurements. We did not use the size threshold of guidelines for the diagnosis of pituitary adenomas on MRI (1). However, studies have shown that, aside from size, MRI quality and the experience of the reader assessing it are both of major importance (13), and, in expert centers, pituitary MRI had excellent diagnostic accuracy for lesions as small as 4 mm (21). Consequently, the performance of imaging studies examined by our multidisciplinary teams cannot be extrapolated to all centers (14). Incidentalomas have been widely described during npCT and incidental pulmonary nodules have been found in up to 50% of scans (22). In contrast, in our series, only 1 false positive of npCT was observed among 155 CD patients and 36 EAS patients. Similarly, the prevalence of npCT false positives was only 3.7% among 215 EAS patients from a systematic review of an EAS series (10). This contrast probably reflects the expertise of the radiologists analyzing the scans, which makes it possible to differentiate various incidentalomas from lesions compatible with an ectopic ACTH-secreting tumor. As mentioned for pituitary MRI, the expertise of the radiologist is of utmost importance and the performance of npCT in our centers cannot be extrapolated to all centers.

Data concerning the high-dose dexamethasone test (HDSST) are lacking in our study because we have long since discontinued

its use. Analysis of the performance of the HDSST revealed disappointing results (5, 17, 23), and 1 large recent study (24) reported that simple clinical and biochemical parameters have a similar diagnostic performance to that of HDSST. Consequently, the test has been abandoned by many expert groups and is not part of the diagnostic algorithm in the pituitary society guidelines (1, 4, 25).

BIPPS, which proved to have excellent diagnostic performance in our series, was performed using CRH injection in the majority of our cases. Its performance may be questioned at a time when CRH is no longer available. However, recent meta-analysis found no difference in the diagnostic performance of BIPSS regardless of the test being associated with desmopressin or CRH (16, 26). Of note, rare false negatives and false positives of BIPSS were observed illustrating that no test (or algorithm) is 100% diagnostic in CS (1, 12).

In conclusion, our study demonstrates the usefulness of classifying patients according to UFC levels for establishing the probability of EAS prior to complementary investigations. This classification, employing a tool used universally in CS, may serve to define a personalized and simplified sequence of investigations aimed at rapidly treating patients with life-threatening EAS (UFC > 10 ULN) while avoiding BIPSS in patients with patent (UFC < 3 × ULN) or highly probable CD. At a time when the CRH test is no longer available, imaging studies have a primary place in the proposed diagnostic strategies after measurement of UFC. This importance will likely be reinforced in the future with the advent of machine learning for the analysis of CT (27), new pituitary imaging processes, and progress in functional imaging (28, 29).

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Data Availability

The data analyzed during the current study are not publicly available out of consideration of intellectual property, and continuing analyses by the study investigators, but may be available from the last author on reasonable request.

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