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Original Research

Bilateral inferior petrosal sinus sampling with human CRH stimulation in ACTH-dependent Cushing's syndrome: results from a retrospective multicenter study

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Abstract

Objective: Bilateral inferior petrosal sinus sampling (BIPSS) is regarded as the gold standard to differentiate between Cushing´s disease (CD) and ectopic Cushing's syndrome (ECS). However, published data on the diagnostic value of additional prolactin analysis are controversial. Thus, we evaluated the diagnostic performance of BIPSS with and without prolactin in a multicenter study.

Design and methods: Retrospective study in five European reference centers. Patients with overt adrenocorticotropin (ACTH)-dependent Cushing's syndrome at the time of BIPSS with human corticotropin–releasing hormone stimulation were eligible. Cut-offs for the inferior petrosal sinus (IPS) to peripheral (P) ACTH ratio and the normalized ACTH:prolactin IPS:P ratio were calculated via receiver operator characteristic analyses (reference: CD).

Results: 156 patients with BIPSS were identified. Of these, 120 patients (92 [77%] females; 106 [88%] CD, 14 [12%] ECS) had either histopathologically confirmed tumors or biochemical remission and/or adrenal insufficiency after surgery; only this subgroup was analyzed by ROC analysis. The optimal cut-offs for the ACTH IPS:P ratio were ≥1.9 at baseline (sensitivity 82.1% [95% CI, 73.2-88.6], specificity 85.7% [95% CI, 56.2-97.5], AUC 0.86) and ≥2.1 at 5 minutes post-CRH (sensitivity 91.3% [95% CI, 83.6-95.7], specificity 92.9% [95% CI, 64.1-99.6], AUC 0.96). A subgroup underwent additional prolactin analysis. An optimal cut-off of ≥1.4 was calculated for the normalized ACTH:prolactin IPS:P ratio (sensitivity 96.0% [95% CI, 77.7-99.9], specificity 100% [95% CI, 56.1-100], AUC 0.99).

Conclusion: Our study confirms the high accuracy of BIPSS in the differential diagnosis of ACTH-dependent Cushing's syndrome and suggests that the simultaneous measurement of prolactin might further improve the diagnostic performance of this test.

Keywords: catheter, Cushing's disease, ectopic, IPSS, petrosal sinus, pituitary, prolactin

Significance

Although bilateral inferior petrosal sinus sampling (BIPSS) is regarded as the gold standard for subtype differentiation of adrenocorticotropin (ACTH)-dependent Cushing's syndrome, published data on its diagnostic accuracy are conflicting. Most historical studies were performed with ovine corticotropin–releasing hormone (CRH), which is not available anymore. In contrast, large-scale analyses on the use of human CRH (which is nowadays commonly used) are still scarce. Here, we report the largest study on BIPSS after stimulation with human CRH, showing that a post-stimulatory cut-off of ≥2.1 allows for the best discrimination between Cushing's disease (CD) and ectopic Cushing's syndrome (ECS). In addition, an explanatory sub-analysis on the usefulness of additional prolactin measurement during BIPSS is provided.

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Introduction

In about 85% of cases, endogenous Cushing's syndrome is due to an inappropriate secretion of adrenocorticotropin (ACTH) that can be caused either by a pituitary adenoma (Cushing´s disease [CD]) or—less likely—by an extra-sellar neuroendocrine tumor (ectopic Cushing's syndrome [ECS]).[1,2] With 80%-85%, CD comprises the vast majority of cases with ACTH-dependent Cushing's syndrome, while ECS is much less prevalent.[1,2]

Imaging procedures alone are usually not able to identify the underlying tumor sufficiently. Firstly, most pituitary and ectopic lesions are rather small (and could therefore easily be overlooked).[3,4] Secondly, inactive pituitary adenomas are present in about 10% of the population,[5,6] possibly leading to the false impression of a pituitary ACTH source at least in some patients with ECS. Endocrine workup is hence a diagnostic cornerstone in patients with ACTH-dependent Cushing's syndrome. However, although basal ACTH levels are usually higher in ECS than in CD, they still show a remarkable overlap between both entities.[7,8] The two most commonly applied dynamic testing procedures (ie, the corticotropin-releasing hormone [CRH] stimulation test and the high-dose dexamethasone suppression test) bear the risk of false-negative results.[9-12] Furthermore, several test combinations do not allow to adequately identify the causative ACTH source.[10,13]

As a consequence, patients with confirmed ACTH-dependent Cushing's syndrome who show discrepancies to imaging and/or equivocal responses to dynamic tests should undergo simultaneous bilateral inferior petrosal sinus sampling (BIPSS).[2,10,11,14] In this test, a relevant gradient between ACTH samples collected centrally (ie, at the inferior petrosal sinus [IPS]) and at a peripheral vein usually reflects CD, whereas comparable ACTH levels are expected in ECS patients.[4,15,16] The use of stimulants (eg, CRH or desmopressin) should further increase the secretion of ACTH from pituitary adenomas (but not from ectopic tumors), thereby improving the diagnostic outcome of the BIPSS. Besides, as an attempt to prevent from false-negative results, some authors suggested simultaneous measurement of prolactin as an indicator of adequate catheterization of the IPS.[17-19] In Europe, however, the historically widely applied stimulant ovine CRH (oCRH) is not available anymore. Human CRH (hCRH) has been described as an appropriate alternative,[20,21] but relatively few data are available to date. In addition, the literature on additional prolactin analysis is controversial.[22]

Consequently, we performed a multicentric analysis in order to evaluate the discriminatory power of hCRH–stimulated BIPSS on a large number of patients with ACTH-dependent Cushing's syndrome. Furthermore, the analytical value of additional prolactin measurement during BIPSS was analyzed in a subset of patients.

Subjects and methods

Participating centers and ethical considerations

This multicenter study was conducted in accordance with the local ethical committees of the five participating centers (the local ethics committee approval numbers were NCH-02-21 in Milan, 152-10 in Munich, 353/2013BO2 in Tübingen, 1457/2016 in Vienna, and 85/12 in Würzburg). All research complied with the Declaration of Helsinki. All patients provided written informed consent to participate to the study.

Subjects

A retrospective cohort of 589 patients with ACTH-dependent Cushing's syndrome was identified via chart review (Tübingen, $n = 167$ [28.4%]; Munich, $n = 149$ [25.3%]; Vienna, $n = 118$ [20.0%]; Würzburg, $n = 108$ [18.3%]; Milan, $n = 47$ [8.0%]). All diagnoses were made according to established criteria between 1988 and 2020.[23] Of note, ACTH levels confirming ACTH dependency were mandatory. Patients with ACTH-dependent Cushing's syndrome who underwent a technically successful BIPSS with hCRH stimulation were considered eligible for the current evaluation (ie, patients with BIPSS under oCRH stimulation were excluded). Center-specific indications for BIPSS included (1) confirmation of a central ACTH source in patients with suspected CD and negative magnetic resonance imaging of the sellar region, (2) confirmation of an ectopic ACTH source in patients with suspected ECS and pituitary abnormalities, and (3) the need for additional diagnostics in case of unequivocal results of dynamic testing procedures. The reference standards of this study were histologically confirmed diagnoses and/or presence of postoperative adrenal insufficiency (patients fulfilling any of these criteria were regarded as members of a "gold standard" cohort, as the respective diagnoses of either CD or ECS were regarded as confirmed).[14]

Bilateral inferior petrosal sinus blood sampling (BIPSS)

The index tests of this study (ie, hCRH–stimulated BIPSS) were performed according to local, comparable protocols. In brief, the catheters were inserted percutaneously into a femoral vein and advanced towards the IPS under application of contrast medium. Depending on the preference of each interventionist, the correct catheter position was documented by digital subtraction angiograph imaging. Blood samples were simultaneously obtained from three ports (a peripheral vein as well as left and right IPS) at −5 and 0 minutes, with the latter sampling directly being followed by injection of 100 μg synthetic hCRH. Afterwards, additional blood samples were taken at 2, 5, 10, 15, and 20 minutes. Of note, the distinct pattern of sampling time points varied slightly from center to center (Table S1).

During BIPSS, patients underwent blood sampling at 0 minutes ($n = 120$), 2 minutes ($n = 56$), 5 minutes ($n = 117$), 10 minutes ($n = 86$), 15 minutes ($n = 65$), and 20 minutes ($n = 19$) (Table S1). Of note, only one center (Vienna) collected samples at 20 minutes (none of their patients had blood sampling at 15 minutes). No significant differences between the sampling time points 15 and 20 minutes were detected during subsequent analyses. Accordingly, the two sampling time points were combined to ≥15 minutes.

The ratio between central and peripheral ACTH values (ie, the ACTH IPS:P ratio) was used for differentiation between CD and ECS. The classical cut-offs for the ACTH IPS:P ratio (ie, ≥2 at baseline and ≥3 after hCRH injection)[15] were compared with newly established cut-offs, evaluating their diagnostic accuracy for differentiation between both sub-entities.

Three centers introduced the additional measurement of prolactin as an attempt to improve the accuracy of the BIPSS. This dataset ($n = 32$) was used to evaluate the diagnostic utility of prolactin for the confirmation of adequate catheterization (which was questioned in case of an ACTH IPS:P ratio < 3 after hCRH, taken a published cut-off into account[17,18]). For this,

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the baseline prolactin IPS:P ratio was calculated ipsilateral to the dominant ACTH IPS:P ratio, with the latter being defined as the highest ACTH IPS:P ratio after administration of hCRH. According to published data, an IPS sampling with a basal ipsilateral prolactin IPS:P ratio of >1.8 was regarded as an adequate catheterization.[17,18] Furthermore, if the basal ipsilateral prolactin IPS:P ratio was ≤1.8, ratios for the normalized ACTH:prolactin IPS:P (being defined as the post-hCRH dominant ACTH IPS:P ratio divided by the basal ipsilateral prolactin IPS:P ratio) of ≥1.3 and ≤0.7 were regarded as specific criteria for CD and ECS.[17] The diagnostic value of these two already published cut-offs and a newly established cut-off for the prolactin-normalized ACTH IPS:P ratio were evaluated in the current patient population.

Biochemical analysis

Plasma ACTH was measured by Siemens Immulite 2000 XPi (Berlin, Tübingen, and Würzburg), Nichols Advantage ACTH assay (Milan), DiaSorin Liaison (Munich), and Roche Cobas (Vienna). Serum cortisol was determined by Siemens Immulite 2000 XPi (Berlin and Würzburg), DiaSorin Liaison (Munich), Siemens ADVIA Centaur XPT (Tübingen), and Roche Cobas (Vienna). In Milan, the Tosoh Bioscience AIA-PACK CORT immunoassay was used until 2016; afterwards, Roche Elecsys was applied. Serum prolactin was analyzed by Siemens Immulite 2000 XPi (Würzburg), Siemens ADVIA Centaur XPT (Tübingen), and Roche Cobas Analyser (Vienna). In Munich, Bayer/Ciba-Corning ACS 180 Plus (until 2010) was applied, followed by Siemens Advia XP (from 2010 onwards).

Statistical analysis

Statistical analysis was performed with SPSS version 26 (IBM Corporation, Armonk, NY, USA) and GraphPad Prism version 8 (GraphPad Software, San Diego, CA, USA). Data are presented as median and interquartile range (IQR). Comparisons between CD and ECS were performed with Mann–Whitney *U*-test for non-normally distributed metrically scaled variables and Pearson's chi-square test for nominally scaled variables. The Kruskal–Wallis test for non-normally distributed metrically scaled variables was used to compare the different centers. The statistical reference test was the Youden's index (J = sensitivity + specificity − 1). This test was used to identify the cut-off balancing best between sensitivity and specificity (using CD as reference) and was chosen because we were convinced that the overall accuracy is more important than maximizing either sensitivity or specificity. Furthermore, Youden's index is considered as a robust tool for the determination of optimal cut-offs.[24,25] To establish optimal cut-offs and their associated sensitivities, specificities, and areas under the curve (AUC), receiver operator characteristics (ROC) curve analyses were performed. Sensitivity, specificity, AUC, positive predictive value, and negative predictive value are always expressed with their 95% confidence intervals (95% CI). Due to the rarity of ACTH-dependent Cushing's syndrome, the need of a confirmed diagnosis (to improve diagnostic accuracy), and the exclusion of BIPSS without hCRH as stimulant, the number of potentially eligible study candidates could not be estimated well. Therefore, our study lacks a formal sample size calculation.

Table 1. Clinical characteristics of patients with confirmed diagnosis ("gold standard" cohort).

	CD	ECS	*P*-value
General characteristics			
Subjects (*n*, %)	106 (88%)	14 (12%)	—
Females (*n*, %)	83 (78%)	9 (64%)	.244
Age (years) (median, IQR)	43 (19)	55 (39)	.100
BMI (kg/m²) (median, IQR)	28 (9)	28 (8)	.691
Source of ACTH-dependent Cushing´s syndrome			
Pituitary gland (*n*, %)	106 (100%)	—	—
Lungs (*n*, %)	—	11 (79%)	—
Pancreas (*n*, %)	—	2 (14%)	—
Paraganglioma (*n*, %)	—	1 (7%)	—
Confirmatory diagnostics			
Histology (*n*)	77	13	.109
Post-operative remission and/or adrenal insufficiency (*n*)	29	1	
Biochemistry			
Basal plasma ACTH (pg/mL) (median, IQR)	56 (48)	103 (100)	.0003
Serum cortisol during the 1 mg DST (μg/d) (median, IQR)	14.2 (11.9)	27.7 (27.7)	.0002
24 h urinary free cortisol (μg/24 h) (median, IQR)	351 (470)	952 (1056)	.0001
Late-night salivary cortisol (μg/dL) (median, IQR)	0.7 (1.1)	1.9 (5.1)	.002
Late-night serum cortisol (μg/dL) (median, IQR)	16.0 (8.2)	29.3 (9.8)	.0007

Of note, basal plasma ACTH was available in 106 CD and 14 ECS patients. Serum cortisol after 1 mg DST was available in 88 CD and 8 ECS patients. 24 urinary free cortisol was available in 97 CD and 12 ECS patients. Late-night salivary cortisol was available in 55 CD and 9 ECS patients. Late-night serum cortisol was available in 29 CD and 5 ECS patients.
ACTH, adrenocorticotropin; CD, Cushing's disease; DST, dexamethasone suppression test; ECS, ectopic Cushing's syndrome; IQR, interquartile range; n.s., not significant.

Results

Baseline characteristics of the study cohort

Out of the entire retrospective cohort of 589 patients with ACTH-dependent Cushing's syndrome, 156 (26.5%) underwent hCRH–stimulated BIPSS. Three of these patients were excluded because the catheterization of the IPS was not bilateral ($n = 2$) or the case had already been reported elsewhere ($n = 1$).[26]

Accordingly, 153 patients remained. Out of this cohort, the tumor subtype was histopathologically confirmed in 90 (57.7%) cases (CD, $n = 77$; ECS, $n = 13$). In 30 (19.6%) additional patients, confirmatory diagnosis was made according to the clinical outcome after surgery (ie, confirmation of biochemical remission and/or temporary adrenal insufficiency). The latter approach allowed identification of 29 cases with CD and one case with ECS. Of note, 33 (21.6%) patients without "gold standard" confirmatory diagnostics were not taken into account for further analyses. The reason for missing confirmatory diagnostics in these patients is outlined in Table S2 while the participant flow diagram is reported in Figure S1.

A detailed description of the 120 "gold standard" patients (CD, $n = 106$ [88%]; ECS, $n = 14$ [12%]) is provided in Table 1. As illustrated, both entities were comparably distributed with respect to age, sex, and body mass index, whereas basal biochemical parameters (ie, ACTH, serum cortisol during the 1 mg overnight dexamethasone suppression test, 24-h urinary free cortisol, and late-night salivary cortisol) were significantly lower in CD than in their ECS counterparts.

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Comparison of conventional and newly generated cut-offs for the ACTH IPS:P ratio during BIPSS

If the conventional cut-off value of ≥2 at baseline was applied, a higher ACTH IPS:P ratio was observed in 86 of 106 CD patients and in two of 14 ECS patients, resulting in a sensitivity of 81.1% (95% CI, 72.1–87.8), and a specificity of 85.7% (95% CI, 56.2–97.5). The conventional cut-off of ≥3 for the post-hCRH ACTH IPS:P ratio was reached by 93 of the 106 CD and two of the 14 ECS patients (sensitivity 87.7% [95% CI, 79.6–93.0], specificity 85.7% [95% CI, 56.2–97.5]).

Of note, only two ECS patients (both with a typical pulmonary neuroendocrine tumor) showed an increased ACTH IPS:P ratio at baseline (and one of these also after stimulation with hCRH).

Figure 1 provides both the scatter plots with the corresponding optimal cut-offs and ROC curves for all time points during BIPSS. At baseline, an optimal cut-off of ≥1.9 for the ACTH IPS:P ratio was calculated. If this cut-off was applied, 87 of 106 CD and 12 of 14 ECS patients were correctly diagnosed (sensitivity 82.1% [95% CI, 73.2–88.6], specificity 85.7% [95% CI, 56.2–97.5], AUC 0.78 [95% CI, 0.74–0.97]) (Table 2). The optimal cut-off for the post-hCRH ACTH IPS:P ratio was ≥2.1 at 5 minutes. This cut-off identified 94 of 103 CD and 13 of 14 ECS patients correctly (sensitivity 91.3% [95% CI, 83.6–95.7], specificity 92.9% [95% CI, 64.1–99.6], AUC 0.96 [95% CI, 0.92–0.99]) (Table 2). If both the basal and the stimulated optimal cut-offs were combined, a sensitivity of 92.2% (95% CI, 84.8-96.3), and a specificity of 85.7% (95% CI 56.2–97.5), respectively, were calculated (Table 2).

A cut-off for the post-hCRH ACTH IPS:P ratio of ≥1.8 at 2 minutes identified 48 out of 49 patients with CD and all ECS ($n = 7$) correctly (sensitivity 98.0% [95% CI, 87.7-99.9] and specificity each 100% [95% CI, 56.1-100], AUC 0.99 [95% CI, 0.98-1.00]). However, as this sampling time point was only conducted in two centers and summarizes only 50% of the entire ECS cohort, the associated results should be interpreted with caution. The ROC analysis of the two sampling time points 10 and ≥15 minutes revealed optimal cut-offs for the post-hCRH ACTH IPS:P ratio of ≥1.5 and ≥1.8. These cut-offs were characterized by sensitivities of 91.0% (95% CI, 81.8–96.0) and 84.2% (95% CI, 73.6–91.2), and specificities of 75.0% (95% CI, 35.6–95.6) and 87.5% (95% CI, 46.7–99.3). If the peak post-CRH ACTH IPS:P value throughout the whole BIPSS was analyzed, an optimal cut-off of ≥2.1 was calculated (sensitivity 94.3% [95% CI, 87.6–97.7], specificity 85.7% [95% CI, 56.1–97.5], AUC 0.92 [95% CI, 0.89–0.99]).

The sensitivities and specificities obtained with the conventional and the newly generated optimal cut-offs for the ACTH IPS:P ratio at baseline and at peak after hCRH stimulation are summarized at the bottom of Table 3.

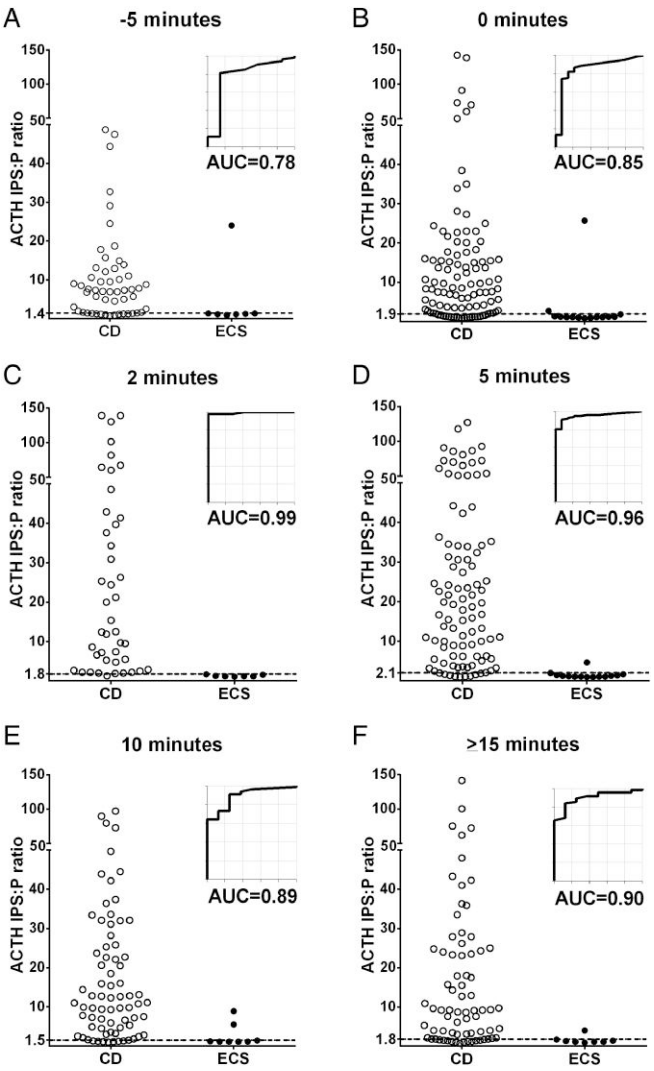

Figure 1. Individual ACTH IPS:P ratios and corresponding ROC curves at different time points during BIPSS. With respect to the scatter plots, the dotted lines illustrate the optimal cut-offs for the ACTH IPS:P ratio at each time point: ≥1.4 at –5 minutes, ≥1.9 at 0 minutes, ≥1.8 at 2 minutes, ≥2.1 at 5 minutes, ≥1.5 at 10 minutes, and ≥1.8 at ≥15 minutes. Few outliers are not reported in the scatter plots: two CD patients at 0 minutes (ratios: 229.5, 321.5), five CD patients at 2 minutes (ratios: 172.4, 195.8, 378.1, 621.2, 651.5, and 2778.0), six CD patients at 5 minutes (ratios: 159.6, 202.5, 202.9, 216.6, 503.9, and 939.3), five CD patients at 10 minutes (ratios: 156.7, 163.7, 252.4, 306.0, and 411.1), and three CD patients at ≥15 minutes (ratios: 157.2, 233.2, and 313.7). Each scatter plot includes the corresponding ROC curve on the upper right. ACTH IPS:P ratio, ratio between ACTH in the inferior petrosal sinus and ACTH in the peripheral blood; AUC, area under the curve; BIPSS, bilateral inferior petrosal sinus sampling; CD, Cushing's disease; ECS, ectopic Cushing's syndrome; IPS, inferior petrosal sinus; P, periphery; ROC, receiver operating characteristics.

Diagnostic value of additional prolactin analysis during BIPSS

BIPSS with concomitant prolactin analysis were available from 32 of our 120 "gold standard" patients (26.6%), involving 25 (78.1%) with confirmed CD and 7 (21.9%) with confirmed ECS. A basal ipsilateral prolactin IPS:P ratio of >1.8 (as suggested elsewhere[17]) was used to confirm adequate catheterization of the IPS.

Out of the entire cohort of 32 patients, 18 (56.3%) cases (CD, $n = 14$; ECS, $n = 4$) were judged as having an adequate catheterization. All of them were correctly identified if the optimal post-hCRH ACTH IPS:P ratio of ≥2.1 at 5 minutes and the basal ipsilateral prolactin IPS:P ratio of >1.8 were applied (Figure 2 and Table S3).

Concerning the remaining 14 patients with a prolactin IPS:P ratio of ≤1.8, two patients with confirmed CD were misdiagnosed as ECS if only the conventional post-hCRH ACTH IPS:P ratio was used. We therefore investigated if the

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Table 2. Comparison between new and conventional cut-offs for bilateral inferior petrosal sinus sampling.

	Cut-off	Sens. (%) (95% CI)	Spec. (%) (95% CI)	Positive predictive value (%) (95% CI)	Negative predictive value (%) (95% CI)
New cut-offs					
Basal ACTH IPS:P ratio[a]	≥1.9	82.1 (73.2-88.6)	85.7 (56.2-97.5)	97.8 (91.4-99.6)	38.7 (42.3-77.6)
Post-hCRH ACTH IPS:P ratio at 5 minutes[b]	≥2.1	91.3 (83.6-95.7)	92.9 (64.1-99.6)	98.9 (93.4-99.9)	59.1 (36.7-78.5)
Combination of both cut-offs[b]	—	92.2 (84.8-96.3)	85.7 (56.2-97.5)	97.9 (92.0-99.6)	60.0 (36.4-80.0)
Conventional cut-offs					
Basal ACTH IPS:P ratio[a]	≥2.0	81.1 (72.1-87.8)	85.7 (56.2-97.5)	97.7 (91.3-99.6)	37.5 (21.7-56.3)
Post-hCRH ACTH IPS:P ratio[b]	≥3.0	87.7 (79.6-93.0)	85.7 (56.2-97.5)	97.9 (91.9-99.6)	48.0 (31.8-71.7)
Combination of both cut-offs[b]	—	90.6 (82.9-95.1)	85.7 (56.2-97.5)	98.0 (92.1-99.6)	54.5 (25.1-67.3)

[a]This analysis included 120 patients. [b]This analysis included 116 patients. ACTH, adrenocorticotropin; AUC, area under the curve; 95% CI, 95% confidence interval; hCRH, human corticotropin–releasing hormone; Sens., sensitivity; Spec., specificity.

Table 3. Overview of the contemporary literature on the diagnostic outcome of the ACTH IPS:P ratio during bilateral inferior petrosal sinus sampling (without time point–specific analyses).

Author (year)	Study cohort (*n*)	Patients with successful BIPSS			Basal ACTH IPS:P			Stimulatory agent	Post-stimulatory ACTH IPS:P		
		Cases (*n*) (rate of all cases [%])	Cases with confirmed CD/ECS (*n*)	Cases with confirmed CD/ECS undergoing stimulation (*n*)	Cut-off	Sens. (%)	Spec. (%)		Cut-off	Sens. (%)	Spec. (%)
Oldfield (1991) (15)	281	278 (98.9%)	215/20	220	2.0	95	100	oCRH	3.0	100	100
Kaltsas (1999) (20)	128	86 (67.2%)	69/6	75	2.0	73	100	oCRH or hCRH or DDAVP	2.0	97	100
Wiggam (2000) (25)[a]	53	NA	44/1	45	2.0 1.5	83 93	100 100	oCRH	NA NA	NA NA	NA NA
Colao (2001) (21)[a]	97	97 (100%)	74/10	84	2.0 2.1	85 85	90 100	oCRH or hCRH	3.0 2.15	88 93	100 100
Swearingen (2004) (26)[a]	179	143 (79.9%)	121/8	83	2.0 NA	85 NA	67 NA	oCRH	3.0 3.3	90 90	67 100
Machado (2007) (27)[a]	56	56 (100%)	50/5	55	2.0 1.45	78 88	100 100	DDAVP	3.0 2.04	92 92	100 100
Castinetti (2007) (28)	42	41 (97.7%)	36/7	43	2.0	86	85	DDAVP	2.0	97	100
Tsagarakis (2007) (29)	54	54 (100%)	47/7	54	2.0	62	100	DDAVP plus CRH[b]	2.0	98	100
Chen (2020) (30)	250	250 (100%)	226/24	250	1.4	95	100	DDAVP	2.8	98	100
Detomas (2023)[a]	156	154 (98.7%)	106/14	120	2.0 1.9	81 82	86 86	hCRH	3.0 2.1	88 94	86 86

[a]These studies are reported twice because they provided results for the conventional cut-off and a newly calculated optimal cut-off. In order to allow for a better comparison, the sensitivities and specificities are always provided without decimal values. [b]Not specified if oCRH or hCRH was used. ACTH, adrenocorticotropin; ACTH IPS:P ratio, ratio between ACTH in the inferior petrosal sinus and ACTH in the periphery; BIPSS, bilateral inferior petrosal sinus sampling; hCRH, human corticotropin-releasing hormone; oCRH, ovine corticotropin-releasing hormone; DDAVP, desmopressin; NA, not available; Sens., sensitivity; Spec., specificity.

additional application of the normalized ACTH:prolactin IPS:P ratio could improve the diagnostic outcome. ROC analysis revealed an optimal cut-off of ≥1.4 for the normalized ACTH:prolactin IPS:P ratio (ie, a ratio of ≥1.4 was regarded as suggestive for CD, whereas ECS was expected in case of a ratio of <1.4). If this cut-off was used in the entire cohort of 32 patients with available data on prolactin, only one patient with confirmed CD was misdiagnosed as ECS, whereas the reminders were correctly identified (sensitivity 96.0% [95% CI, 77.7-99.9], specificity 100% [95% CI, 56.1-100], AUC 0.99 [0.96-1.00]) (Figure 3). More details on the cohort undergoing additional prolactin measurement during BIPSS are reported in Table S3.

Furthermore, we also evaluated the outcome of cut-offs for the normalized ACTH:prolactin IPS:P ratio that were formerly suggested by Sharma et al.[17] (Figure S2). For a better

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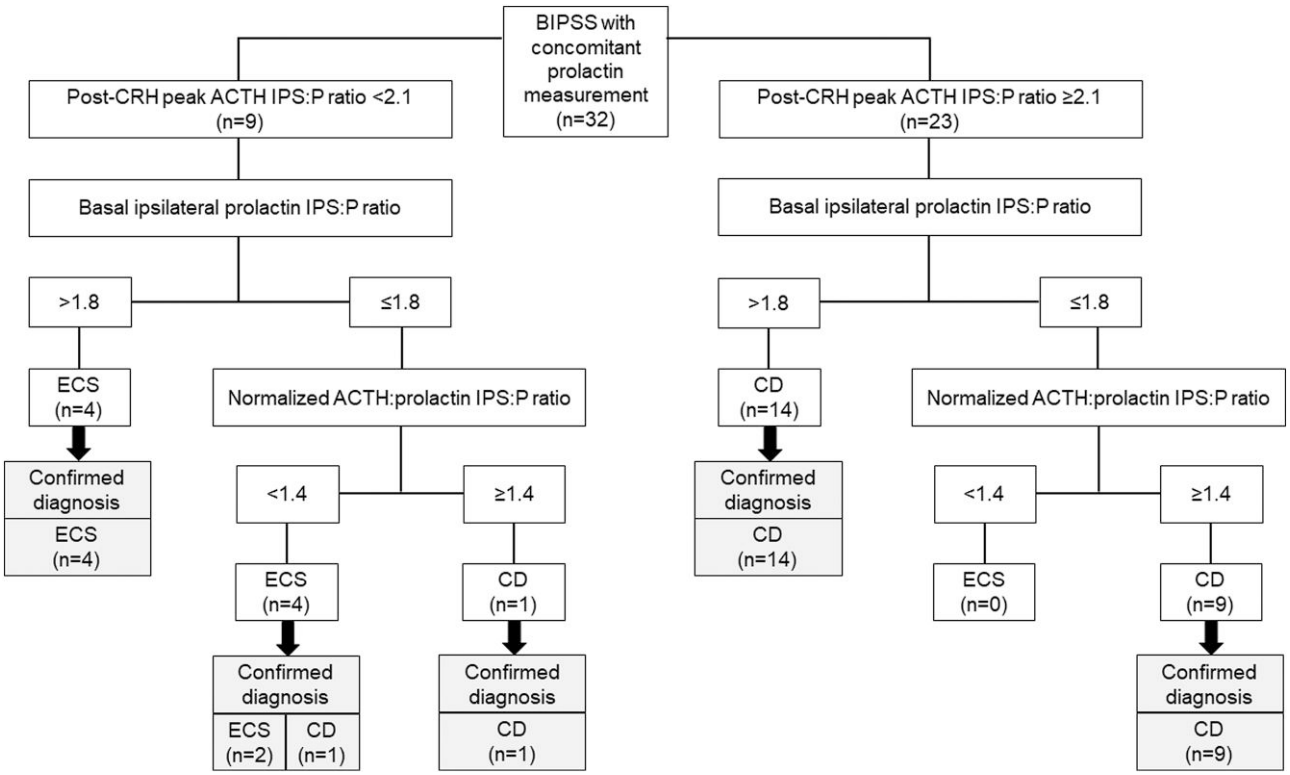

Figure 2. Diagnostic value of the normalized ACTH:prolactin IPS:P ratio in case of uncertain catheterization during BIPSS. Ipsilateral prolactin ratio corresponds to the dominant inferior petrosal sinus ACTH level. The cut-off ratio of 1.8 (with lower values being suggestive for uncertain catheterization) was reported in two studies.[17,18] ACTH, adrenocorticotropin; ACTH:prolactin IPS:P ratio, normalized ratio between the highest ACTH level in the inferior petrosal sinus and the periphery, with the ipsilateral basal prolactin in the inferior petrosal sinus and the periphery; IPS, inferior petrosal sinus; P, periphery.

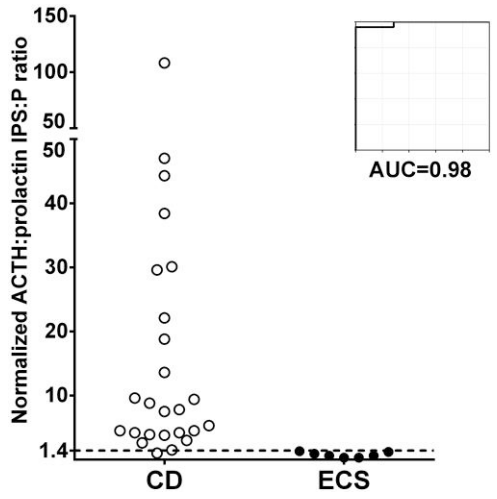

Figure 3. Individual normalized ACTH:prolactin IPS:P ratios during BIPSS and corresponding ROC curves. The dotted line illustrates the optimal cut-off of ≥1.4 for the ACTH:prolactin IPS:P ratio. ACTH, adrenocorticotropin; BIPSS, bilateral inferior petrosal sinus sampling; IPS, inferior petrosal sinus; P, periphery; ROC, receiver operating characteristics.

comparison of the two studies, a post-hCRH ACTH IPS:P ratio of ≥3 was used (with higher values indicating presence of CD). Applying the historical cut-off of ≥1.3 for the diagnosis of CD, one of our two patients with confirmed ECS would have been erroneously diagnosed as CD (due to a normalized ACTH:prolactin IPS:P ratio of 1.3). In contrast, none of our patients had a normalized ACTH:prolactin IPS:P ratio of ≤0.7 (indicating ECS). Sharma et al.[17] also proposed a "gray area" (for ratios between 0.8 and 1.2) in which the diagnosis is regarded as uncertain. With respect to our cohort, one single CD patient (with a normalized ACTH:prolactin IPS:P ratio of 1.0) and two ECS patients (with normalized ACTH:prolactin IPS:P ratios of 1.2 and 0.9) were within this "gray area."

Discussion

BIPSS is considered as the "gold standard" for differentiating CD and ECS. We here report the results of a large European multicenter analysis with a well-characterized study cohort. In comparison to the published "conventional" thresholds, our new cut-offs for both the basal and the post-hCRH ACTH IPS:P ratio increased sensitivity from 81.1% to 82.1%, and from 87.7% to 91.3%. In contrast, an increase in specificity was only found after stimulation with hCRH (from 85.7% to 92.9%). Besides, our current results support the diagnostic value of additional prolactin analysis (as outlined by a sensitivity of 96.0%, and a specificity of 100%).

The "conventional" cut-off of ≥2 (before stimulation) for the ACTH IPS:P ratio was proposed by Oldfield et al. in 1991[15] and repeatedly applied since then.[20,21,27-31] With respect to our current cohort, this baseline cut-off resulted in a sensitivity of 81.1%, and a specificity of 85.7% (what is well in line with formerly reported data, as shown in Table 3). Our new baseline cut-off of ≥1.9 slightly improved sensitivity to 82.1%, while specificity remained unchanged (indicating moderate diagnostic benefits of our new cut-off). Three other groups suggested lower baseline cut-offs (ranging

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from 1.4 to 1.5).[27,29,32] The reported sensitivities (ranging from 88% to 95%) and specificities (always at 100%) were higher than ours. In one case, the reason for this discrepancy is probably related to the use of desmopressin (DDAVP) as stimulant.[32] In the other two studies, however, the causative effect remains speculative. Nevertheless, no diagnostic test will be 100% accurate and we therefore have the impression that our current data more reliably reflect real-world settings.

A post-stimulatory cut-off of ≥3 for the post-stimulatory ACTH IPS:P ratio during BIPSS was also firstly suggested by Oldfield et al.,[15] applying oCRH as stimulant. Since then, several other studies on the diagnostic performance of BIPSS proposed numerous protocol modifications (particularly with respect to the stimulants applied) and variable cut-offs (as outlined in Table 3). Of note, it has been indicated that oCRH results in a prolonged and more pronounced response of both ACTH and cortisol,[33,34] whereas comparable effects to hCRH were reported by others.[35,36] A direct comparison between the two compounds would certainly be of interest; however, the latter is not commercially available any more (at least in Europe).

In our current study, the conventional cutoff of ≥3 for the post-hCRH ACTH IPS:P ratio at 5 minutes yielded a sensitivity of 87.7%, and a specificity of 85.7%. Our new optimal cut-off of ≥2.1 5 minutes after hCRH stimulation showed the highest discriminatory power of all sampling time points during BIPSS (as outlined by a significant increase of sensitivity and specificity to 91.3% and 92.9%). Of note, the combination of both optimal cut-offs (ie, the basal and the post-hCRH equivalent) resulted in a slight increase in sensitivity (92.2%) but a significant decrease in specificity (85.7%). If our optimal cut-off of ≥2.1 for the peak post-CRH ACTH IPS:P value was compared with the conventional cut-off of ≥3.0, an increase in sensitivity was identified (94.3% vs 87.7%) whereas specificity was identical (85.7%).

Our newly obtained optimal cut-off of ≥2.1 after hCRH stimulation (that was calculated for both the post-CRH time point at 5 minutes and the peak post-CRH ACTH IPS:P value throughout the whole test) is very close to the cut-off of ≥2.0 reported by Kaltsas et al.[20] In the latter study, most patients underwent stimulation with hCRH (only few patients were stimulated with oCRH). However, it is important to highlight that no ECS patient was regarded as false positive if a cut-off of ≥2.0 for the ACTH IPS:P ratio was applied (either with or without stimulation). A possible explanation for this finding is the low number of ECS ($n = 6$; with only five of them undergoing any CRH stimulation).

An optimal cut-off of 2.15 was reported by Colao et al.[21] in a study in which either oCRH or hCRH were used as stimulants. With respect to our own cohort, we identified post-CRH ACTH IPS:P ratios of 1.9 and 2.0 in two CD patients, and of 2.0 in one ECS patient.

Subsequently, Swearingen et al.[28] proposed an optimal cut-off of 3.3 for the post-oCRH ACTH IPS:P ratio. Sensitivity and specificity were particularly high (ie, 89.7% and 100%); however, the number of patients with confirmed ECS undergoing oCRH stimulation was again very limited ($n = 3$).

Some studies reported the use of desmopressin as stimulatory agent.[29,30,32] The cut-offs reported by Machado et al.[29] and Castinetti et al.[30] were significant lower than the post-stimulatory cut-off of ≥3 originally reported by Oldfield et al.[15] On the other hand, a more recent and larger study on desmopressin stimulation during BIPSS postulated a post-stimulatory cut-off of 2.8 which is well comparable to the original cut-off.[32] Furthermore, the recent data is also in good accordance with a study where both secretagogues showed a comparable performance.[37] Interestingly, when Tsagarakis et al.[31] conducted simultaneous stimulation with CRH and desmopressin, they observed remarkably high sensitivity (97.9%) and specificity (100%). Nonetheless, it is worth mentioning that the size of their entire study cohort was limited as well ($n = 54$).

By validating the cut-offs reported in the literature in our population, the cut-off of ≥2.0 resulted in higher sensitivity, while specificity dropped to 78.6% (of note, none of the other formerly published optimal cut-offs resulted in higher specificity). We therefore have the impression that a cut-off of >2.0 can be used conventionally (thereby also improving clinical practicability). However, values close to 2.0 have to be interpreted with caution, and BIPSS should eventually be repeated. The obtained sensitivity and specificity are slightly different from those reported in a recent metanalysis involving 1642 patients.[38] The difference in sensitivity (91.2% vs 94%) and specificity (92.9% vs 89%) compared to our study may be explained by the fact that the metanalysis considered studies with any stimulatory agent (i.e, oCRH, hCRH, and DDAVP), while we investigated BIPSS with hCRH stimulation only.

Of note, only one patient was incorrectly diagnosed with the newly generated optimal cut-off of ≥1.8 for the ACTH IPS:P ratio at 2 minutes. This result, however, has to be interpreted with caution as there were only 56 (CD $n = 49$; ECS $n = 7$) evaluable patients at this time point. Further studies may elucidate the diagnostic potency of the sampling time point 2 minutes after stimulation.

Another important finding of this study is related to the duration of BIPSS. In our series, sensitivity and specificity decreased if sampling time points of more than 5 minutes post-hCRH were evaluated. Our observation that the best discriminatory effect of ACTH occurs within the first 5 minutes after stimulation has already been described by others.[20,39] In comparison to previous publications, however, only one of the nine false-negative CD patients at 5 minutes was correctly identified later than 10 minutes from hCRH stimulation. This patient had a stimulated ACTH of 1.8 at 15 minutes, while the ACTH values at other sampling time points were lower than the respective cut-offs. Nevertheless, considering the invasiveness of BIPSS, a test duration of more than 5 minutes after stimulation may expose patients to additional risks without substantial improvement of the diagnostic outcome.

In order to prove adequate catheterization of the IPS and to strengthen the diagnostic outcome, some studies[17,18] proposed the additional measurement of prolactin during BIPSS. In specific, the basal ipsilateral prolactin IPS:P ratio was suggested for confirmation of adequate cannulation of the IPS, whereas the normalized ACTH:prolactin IPS:P ratio was proposed for differentiation of CD and ECS. In our study, we observed that the additional analysis of these parameters increased the diagnostic accuracy of BIPSS. We observed only one misclassified patient if our new optimal cut-off of ≥1.4 for the normalized ACTH:prolactin IPS:P ratio was applied. Nevertheless, although the current study represents one of the largest cohorts on this topic to date, a larger evaluation on the diagnostic value of additional prolactin analysis during BIPSS (in general) and our newly generated optimal

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cut-off of ≥1.4 for the normalized ACTH:prolactin IPS:P ratio (in particular) is certainly required. This is especially true as a consistent co-lateralization of prolactin drainage (with the consequence of diminished diagnostic accuracy in CD in case of prolactin correlation) was described more recently.[22]

The present study has certainly some limitations. Apart from its retrospective and multicenter nature, the number of patients (especially with ECS) and consequently also the statistical power of the study are limited. Furthermore, our study also lacks a formal sample size calculation. Despite these limitations, we here report not only one of the largest cohorts on this topic ever published but also the largest series of BIPSS undergoing hCRH stimulation (with the latter being the only commercially available CRH agent to date). Besides, our multicentric study (that aimed for an optimal single cut-off for the outcome interpretation of BIPSS) may have generated more realistic (ie, not too optimistic) results.

Compared to the conventionally applied cut-offs, our new cut-offs allow for a significant increase in sensitivity. Considering both the minor additional diagnostic value and the risks related to a prolonged BIPSS, an extension beyond 5 minutes after stimulation does not appear justified. Furthermore, the analysis of the normalized prolactin:ACTH IPS:P ratio seems to be a promising factor by increasing the diagnostic outcome of BIPSS.

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Declaration of interests

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Supplementary material

Supplementary material is available at *European Journal of Endocrinology* online.

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