

1 **Supplemental Data**

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3 **Prolactin levels – at baseline and post-CRH stimulation**

4 Baseline peripheral prolactin levels were normal in all occult and EAS patients. Four
5 patients with Cushing’s disease had mildly elevated baseline peripheral prolactin levels of 31.9–
6 45.9 ng/ml (1.4–2.0 nmol/liter), normal < 20 ng/ml (< 0.9 nmol/liter) with no significant change
7 after CRH. Prolactin immunostaining was not performed on resected tissues. Baseline IPS
8 prolactin levels on the dominant side were 11.1-498.6 ng/ml (0.5-21.7 pmol/liter) in patients with
9 Cushing’s disease and 4.2-56.2 ng/ml (0.2-2.4 pmol/liter) in EAS. Baseline IPS prolactin levels
10 were higher on the side of the tumor in 9 (inter-petrosal prolactin gradient = 1.4-4.9), higher on
11 the opposite side in two (inter-petrosal prolactin gradient = 1.3, 4.1) and similar on the two sides
12 in 5 of the 17 Cushing’s disease patients. After CRH, 10 (59%) patients with Cushing’s disease
13 had greater than 10% increase in prolactin levels while six (35%) had greater than 10% decrease
14 on the side with the dominant ACTH IPS/P. Six EAS patients (75%) had greater than 10%
15 increase while two (25%) had greater than 10% decrease in values on the dominant side. The
16 change in prolactin did not parallel the change in ACTH levels post-CRH stimulation.

17 Post-CRH prolactin IPS/P ratios on the dominant side were > 1.8 in the seven EAS
18 patients with true negative IPSS results. However, use of post-CRH prolactin values to calculate
19 the normalized ACTH IPS/P ratios led to indeterminate results (0.6–0.7) in three cases while it
20 was 5.3 in the false positive case (Supplemental Table 3).

21 Post-CRH prolactin IPS/P ratios in 15 true positive Cushing’s disease patients were > 1.8.
22 One patient with true positive IPSS result and evidence of successful catheterization using
23 baseline prolactin values had a post-CRH prolactin IPS/P ratio of 1.6; normalized ACTH/post-

24 CRH prolactin IPS/P ratio was 56.1. The Cushing's disease patient with false negative IPSS
25 result was correctly identified using both baseline and post-CRH prolactin values (Supplemental
26 Table 3).

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43 **Supplemental Table 1:** IPSS ACTH and prolactin data pre (-5 and 0 minutes) and post-CRH stimulation
 44 (+3, +5, and +10 minutes) in a patient with occult Cushing's syndrome (Occult 2).

Time	ACTH (pg/ml)					Prolactin (ng/ml)				
	Right IPS	Left IPS	P	Right IPS/P	Left IPS/P	Right IPS	Left IPS	P	Right IPS/P	Left IPS/P
-5	106	96	87	1.2	1.1	72.7	17.0	19.7	3.7	0.9
0	102	93	88	1.1	1.1	71.6	14.5	17.1	4.2	0.8
3	188	89	82	2.3	1.1	90.9	16.1	15.0	6.1	1.1
5	139	82	85	1.7	1.0	82.6	14.4	16.6	5.0	0.9
10	138	88	81	1.7	1.1	52.0	15.1	14.2	3.7	1.1

45 IPS = inferior petrosal sinus, IPS/P = inferior petrosal sinus to peripheral ratio, P = peripheral. To convert
 46 ACTH level to pmol/liter, multiply by 0.2202. To convert prolactin levels to nmol/liter, multiply by
 47 0.04348; prolactin levels in ng/ml are equivalent to mcg/liter.

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59 **Supplemental Table 2:** IPSS venogram results and baseline prolactin IPS/P ratios in patients with
60 ACTH-dependent Cushing's syndrome (patients with normal venous anatomy and baseline prolactin
61 IPS/P > 1.8 {n = 14} are not shown in the table)

Anomalous IPSS venous anatomy/drainage			
Baseline PRL IPS/P ≤ 1.8			
Type	IPSS result	Baseline PRL IPS/P	Venogram
Occult 4	-	1.6	Large right and small left petrosal vein
Occult 3	-	1.2	Small left petrosal vein
CD	FN	1.0	Large left and somewhat smaller right petrosal vein, catheter could not be progressed fully into the right petrosal vein
Baseline PRL IPS/P > 1.8			
Type	IPSS result	Baseline PRL IPS/P	Venogram
CD	TP	6.2	Large left and small right petrosal vein
CD	TP	5.7	Very attenuated communicating veins between cavernous sinus and inferior petrosal vein- sampling done from expected draining veins
CD	TP	4.7	Inferior petrosal veins joined an anterior separate jugular division that then joined the main vein at the mandible, larger right and narrower left petrosal vein
CD	TP	10.9	Hypoplastic, narrow left petrosal vein. Smaller catheter used
Ectopic	TN	3.9	Right jugular vein divided early sending a branch to draining cavernous sinus and to vertebral plexus and a second branch to base of skull draining the sigmoid and transverse sinus; left petrosal vein narrow, no difficulty in cannulation
CD	TP	2.2	Right inferior petrosal sinus drained into both the right jugular and right vertebral plexus, predominant drainage into the right vertebral plexus, otherwise normal
Occult 2	-	4.2	Bilateral small petrosal veins - difficult to cannulate
CD	TP	2.9	Generous right petrosal vein and narrow left petrosal vein, able to cannulate with 2.8 French catheter
Normal venous anatomy/drainage			
Baseline PRL IPS/P ≤ 1.8			
Type	IPSS result	Baseline PRL	Venogram

		IPS/P	
CD	TP	1.1	Normal
Ectopic	FP	1.7	Normal
CD	TP	0.8	Normal
Procedural difficulty during IPSS			
Type	IPSS result	Baseline PRL IPS/P	Venogram
CD	TP	3.4	Tortuous course of left innominate vein, left petrosal vein catheter unstable and moved out, samples delayed by 2 min due to repositioning, venous anatomy normal

62 CD = Cushing's disease, PRL = Prolactin, IPS/P = inferior petrosal sinus to peripheral ratio, TP = true
63 positive, TN = true negative, FP = false positive, FN = false negative

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80 **Supplemental Table 3:** IPSS ACTH (pg/ml) and prolactin (ng/ml) data in 29 patients with ACTH-
81 dependent Cushing’s syndrome with pre and post-CRH prolactin IPS/P ratios and normalized ACTH
82 IPS/P ratios using both pre and post-CRH prolactin values.

Type	IPSS result	Peak ACTH IPS/P	Ipsilateral BL PRL IPS/P	Normalized Peak ACTH/BL PRL IPS/P	Ipsilateral Peak PRL IPS/P	Normalized Peak ACTH/Peak PRL IPS/P
EAS	TN	1.2	1.8	0.7	2.1	0.6
EAS	TN	1.5	3.1	0.5	2.3	0.7
EAS	TN	1.1	4	0.3	4.5	0.3
EAS	TN	1.4	5.4	0.3	9.0	0.2
EAS	TN	1.1	4.8	0.2	4.3	0.3
EAS	TN	1.2	4.7	0.3	9.7	0.1
EAS	TN	1.4	3.9	0.4	2.1	0.7
EAS	FP	9.5	1.7	5.6	1.8	5.3
CD	TP	20.4	4.7	4.3	7.3	2.8
CD	TP	113.4	10.9	10.4	4.9	23.2
CD	TP	53.6	5	10.8	5.7	9.5
CD	TP	131.7	6.2	21.4	3.0	43.5
CD	TP	20	2	10.1	2.2	9.3
CD	TP	49.2	5.7	8.6	3.8	12.9
CD	TP	87.5	2.1	40.9	1.6	56.1
CD	TP	9.7	0.8	11.6	2.5	3.9
CD	TP	18.5	3.6	5.1	5.1	3.6
CD	TP	167.3	4.7	35.5	30	5.6
CD	TP	37.7	2.2	16.8	2.8	13.3
CD	TP	36.3	10.9	3.3	4.2	8.7
CD	TP	9.2	1.1	8.4	1.9	5.0
CD	TP	11.3	2.9	3.9	6.2	1.8
CD	TP	40.9	3.4	11.9	3.4	12.2
CD	TP	78.2	3.1	24.9	3.9	19.9
CD	FN	1.3	1	1.3	1.0	1.3
Occ 1	--	1.3	13.2	0.1	11.7	0.1
Occ 2	--	2.3	4.2	0.6	6.1	0.4
Occ 3	--	1.3	1.2	1.1	1.4	1.4
Occ 4	--	1.4	1.6	0.9	0.7	0.7

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