Supplemental Online Content

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This supplemental material has been provided by the authors to give readers additional information about their work.
## Methods

Specific protocol approvals were from the National Institute Child Health and Human Development (NICHD) and National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Institutional Review Boards. Ectopic adrenocorticotropin hormone secretion (EAS) was likely if inferior petrosal sinus sampling (IPSS), 8 mg dexamethasone suppression and/or Corticotropin Releasing Hormone (CRH) stimulation tests were consistent with the diagnosis.\(^1\) Such patients underwent additional testing for tumor markers at the endocrinologist's discretion, including urine 5-hydroxyindoleacetic acid (5-HIAA), serum gastrin, plasma and urine catecholamines and metabolites and/or serum calcitonin. Imaging studies identifying the source of EAS included computed tomography (CT) and/or magnetic resonance imaging (MRI) scans of the neck, chest, abdomen, and pelvis. Some patients also underwent scintigraphy with 6 mCi octreotide, 18 mCi octreotide, \(^{18}\text{F}\)-DOPA positron emission tomography (PET)/CT, and/or Gallium-68 Dotatate PET/CT.

Surgical techniques included posterolateral thoracotomy, anterior thoracotomy, and video-assisted thoracoscopic surgery (VATS). The goal of operation was to resect all gross disease for negative margins whenever practicable. Post-operatively, morning serum cortisol, Adrenocorticotropic Hormone (ACTH) and 24-hour urinary cortisol levels were measured. Patients were considered disease-free if the post-operative serum cortisol level was below 5 \(\mu\text{g/dL}\) in those who were hypercortisolemic at the time of surgery. Adrenalectomized patients were deemed cured if ACTH decreased to normal adrenalectomized levels and suppressed after 8 mg dexamethasone. Eucortisolemic patients receiving preoperative steroidogenesis inhibitors were considered cured if they had normal 1 mg dexamethasone suppression and a normal cortisol diurnal rhythm. After surgery, patients with persistent endocrinopathy continued follow-up and laboratory/imaging testing to identify EAS.

Tumors were reviewed by pathologists, with most specimens evaluated for ACTH by immunohistochemistry (IHC). Histologic classification as Atypical Carcinoid (AC) was based on World Health Organization (WHO) criteria,\(^2\) with any of these features: increased mitotic rate, pleomorphism, irregular nuclei, or areas of necrosis. Otherwise, tumors were classified as Typical Carcinoid (TC) if none of those features were present. Staging was...
updated according to the 8th edition of the American Joint Committee on Cancer (AJCC) system for lung cancer. Tumors without nodal evaluation were categorized as NX. Follow-up information including recurrence, adjuvant treatments, and survival status was identified from the medical record at the last clinical visit or contact and the Social Security Death Index.

For the Kaplan-Meier analysis, patients who completed lung surgery but had ongoing hypercortisolism/ excessive ACTH are defined as persistent and treated as being failures at time zero in the Disease-Free Survival (DFS) curves (i.e., zero duration of being disease-free), while patients who completed lung surgery with no residual hypercortisolism/ excessive ACTH but later redeveloped Cushing’s Syndrome (CS) are defined as recurrent and treated as a failure for DFS at the time of recurrence.
**eResults**

*Baseline Characteristics*

Prior to presenting, few patients had undergone an endocrine procedural intervention with 11/68 (16%) adrenalectomies and 8/68 (12%) hypophysectomies.

*Imaging Studies*

Chest CT and MRI localized the target pulmonary lesion(s) in 62/68 (91.2%) and 49/61 (80.3%) of patients, respectively. Of the patients with CS and known pulmonary lesions on imaging, 12/65 (18.5%) of patients also had an abnormal pituitary MRI. Since 2002, PET-based modalities have been used routinely to identify a pulmonary source of CS. Dotatate and $^{18}$F-DOPA PET/CT imaging detected lung lesions in 13/20 (65.0%) and 23/37 (62.2%) of patients respectively. Adrenal gland nodules were also noted in 6/60 (10.0%) of patients by abdominal CT imaging. The adrenal lesions were present in the background of bilateral adrenal hyperplasia, and none proved to be ACTH-secreting.

*Pathology*

The major histologic type was TC (57/68, 83.8%). Tumors were characteristically small with an average diameter of 1.1 cm (range, 0.1-3.5 cm). Of patients with at least N1 nodal staging, 22/59 (37.3%) had nodal involvement, and stratified by histopathology, 15/49 (30.6%) of TC had nodal disease while 7/10 (70.0%) of AC had nodal disease. Lymphatic or vascular invasion was found in 11/68 (16.2%) of all resected tumors with 10 of them being AC.

*Persistent/Recurrent Disease Patterns and Treatments*

Four of seven patients with recurrent tumor underwent adrenalectomy. Six of seven had additional surgical therapy for lung resection with repeated lymph node dissection when indicated, with sub-lobar resection employed in 1/7 of patients. Complete lymph node assessment was performed in 5/7.
with 2/7 having a partial nodal assessment (N1 only). Pathologic nodal disease was found in 4/7, including the lone patient with an AC tumor.

Regarding the location of the recurrent tumors, disease occurred locoregionally in 5/7, the contralateral chest in 1/7, or locoregional and distant in 1/7.

Of patients with persistent disease, sub-lobar resection was used in 2/4, with complete nodal dissection performed in 3/4 that yielded negative findings. Persistence of disease after surgery was definitively treated with bilateral adrenalectomy in 1/4, redo-sub-lobar/nodal resection in 1/4, bilateral adrenalectomy plus redo-chest/nodal dissection in 1/4, or medical management in 1/4 until further work up and definitive redo therapy is planned.

Adjuvant therapy was used on a case-by-case basis, including radiation therapy (in a total of 6 patients from our first report⁴), medical therapy to reduce cortisol production (ketoconazole, aminogluthethimide, cabergoline, metyrapone, and/or mitotane), and repeat surgical resections in the chest or bilateral adrenalectomy per the treating physicians.
References

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### eTable1: Demographic Variables and Pre-operative Evaluations

| Variable                                      | n (%)         |
|-----------------------------------------------|---------------|
| **Age (years): median (range)**               | 41 (17-80)    |
| Male                                          | 29/68 (42.6)  |
| **FEV₁ (%)**: median (range)                  | 88 (31-116)   |
| **Tobacco Use**                               |               |
| Current/History of Use                        | 9/30 (30.0)   |
| Never                                         | 21/30 (70.0)  |
| Unknown                                       | 38/68 (55.9)  |
| **Race**                                      |               |
| White                                         | 54/66 (81.8)  |
| Black                                         | 8/66 (12.1)   |
| Hispanic                                      | 4/66 (6.1)    |
| Unknown                                       | 2/68 (2.9)    |
| **Number of patients treated per decade**     |               |
| 1980s                                         | 14/68 (20.6%) |
| 1990s                                         | 15/68 (22.1%) |
| 2000s                                         | 16/68 (23.5%) |
| 2010s                                         | 21/68 (30.9%) |
| 2020s                                         | 2/68 (2.9%)   |
| **Clinical Presentation**                     |               |
| Cushing’s Syndrome                            | 68/68 (100)   |
| Bronchial symptoms                            | 0/68 (0)      |
| **Pre-operative Imaging**                     |               |
| Chest CT                                      | 68/68 (100)   |
| Pituitary MRI                                 | 65/68 (95.6)  |
| Chest MR                                      | 61/68 (89.7)  |
| Abdominal CT                                  | 60/68 (88.2)  |
| Abdominal MR                                  | 53/68 (77.9)  |
| Octreotide scintigraphy                       | 42/68 (61.8)  |
| ¹⁸F-DOPA PET/CT                               | 37/68 (54.4)  |
| Gallium-68 Dotatate PET/CT                    | 20/68 (29.4)  |
| **Prior Surgical Interventions**              |               |
| Adrenalectomy                                 | 11/68 (16.2)  |
| Hypophysectomy                                | 8/68 (11.8)   |

*FEV₁: Forced Expiratory Volume in 1 second*
| Symptom                                      | n (%)       |
|----------------------------------------------|-------------|
| Fatigue/Muscle weakness                      | 58/68 (85.3)|
| Hypertension                                 | 56/68 (82.3)|
| Hirsutism                                    | 28/39 (71.8)|
| Moon facies                                  | 44/68 (64.7)|
| Truncal adiposity                            | 44/68 (64.7)|
| Striae (violaceous)                          | 41/68 (60.3)|
| Psychiatric disorders                        | 38/68 (55.9)|
| Bruising                                     | 37/68 (54.4)|
| Edema                                        | 35/68 (51.5)|
| Menstrual irregularity                       | 17/39 (43.6)|
| History of Diabetes Mellitus                 | 26/68 (38.2)|
| Obesity (Body Mass Index > 30kg/m²)          | 14/44 (31.8)|
| Infections                                   | 21/68 (30.9)|
| Hyperpigmentation                            | 19/68 (27.9)|

*aBody Mass Index not recorded in 24/68 patients*
### eTable 3: Wedge/Segmentectomy Only Patient Characteristics

| Patient (year) | Age/Sex | FEV1(%) | Stage | Index Surgery, Extent of nodal dissection | Typical/Atypical | Persistent/Recurrence | Time to Recurrence (months) | Follow-up Months | Status at Last Follow-up |
|----------------|---------|---------|-------|------------------------------------------|------------------|-----------------------|------------------------------|-----------------|------------------------|
| 1 (1983)       | 28/F    | IA1     | Wedge | Typical                                  | Persistent elevation in ACTH and UFC | None                  | NA                           | 2               | Dead with persistent disease |
| 2 (2003)       | 55/M    | IA1     | Wedge, N1+N2 | Typical | None | NA | 0.5 | Alive, tumor free |
| 3 (2006)       | 68/F    | IA1     | Wedge, N1+N2 | Atypical | None | NA | 0.5 | Alive, tumor free |
| 4 (2007)       | 22/F    | IIIA    | Segment, N1+N2 | Typical | Recurrent, ipsilateral lung | None | 36 | 143 | Alive with disease |
| 5 (2009)       | 52/F    | IA1     | Wedge, N1+N2 | Typical | None | NA | 24 | Alive, tumor free |
| 6 (2009)       | 62/M    | T1aNX   | Wedge | Typical | None | NA | 108 | Alive, tumor free |
| 7 (2009)       | 55/F    | T1aNX   | Wedge | Typical | None | NA | 21 | Alive, tumor free |
| 8 (2010)       | 60/M    | T1bNX   | Wedge | Typical | None | NA | 0.5 | Alive, tumor free |
| 9 (2010)       | 59/M    | T1bNX   | Wedge | Typical | None | NA | 1 | Alive, tumor free |
| 10 (2010)      | 58/F    | IA2     | Wedge, N1+N2 | Atypical | None | NA | 9 | Alive, tumor free |
| 11 (2011)      | 30/M    | T1bNX   | Wedge | Typical | None | NA | 17 | Alive, tumor free |
| 12 (2011)      | 51/F    | T1aNX   | Wedge | Typical | None | NA | 13 | Alive, tumor free |
| 13 (2013)      | 67/F    | IA1     | Wedge, N1+N2 | Typical | None | NA | 13 | Alive, tumor free |
| 14 (2016)      | 55/F    | T1aNX   | Wedge | Typical | None | NA | 25 | Alive, tumor free |
| Case No. | Age/Gender | Stage | Location | B-Stage | Lymph Nodes | Follow-up | Status |
|----------|------------|-------|----------|---------|-------------|-----------|--------|
| 15 (2017)| 64/M       | IIB   | Segment, N1+N2 | Typical | None        | NA        | 3      |
| 16 (2017)| 80/F       | IA2   | Wedge, N1+N2   | Typical | Persistent, contralateral lung and mediastinum | NA        | 26     |
| 17 (2018)| 17/F       | IIB   | Segment, N1+N2 | Typical | None        | NA        | 0.1    |
| 18 (2018)| 22/M       | IA1   | Segment, N1+N2 | Typical | None        | NA        | 14     |
| 19 (2019)| 56/F       | IA2   | Wedge, N1+N2   | Atypical | None        | NA        | 0.5    |

*M = Male, F = Female, UFC = urine free cortisol, NX = Nodes not sampled, FEV₁ = Forced Expiratory Volume in 1 second*
| Patient (year of surgery) | Age/Sex | Time to Surgery (months) | Stage | Index Surgery, Extent of nodal dissection | Typical/Atypical | Recurrent | Time to Recurrence (months) | Additional Therapy | Follow-up Months | Status at Last Follow-up |
|--------------------------|---------|--------------------------|-------|------------------------------------------|-----------------|-----------|----------------------------|-------------------|------------------|------------------------|
| 1 (1989)                 | 45/F    | 1                        | IA1   | Lobectomy, N1+N2                         | Typical         | Recurrent, contralateral chest, ipsilateral hilum | 152               | None reported | 234                  | Dead with cancer   |
| 2 (1990)                 | 28/M    | 1                        | IA1\textsuperscript{a} | Lobectomy, N1 only                       | Typical         | Recurrent, ipsilateral hilum | 45               | Ketoconazole, amoinogluethimide, bilateral adrenalectomy, RT | 87                | Alive with disease |
| 3 (1990)                 | 30/F    | 3                        | IIB\textsuperscript{a} | Lobectomy, N1 only                       | Typical         | Recurrent, ipsilateral hilum | 114              | Thoracotomy and nodal dissection, ketoconazole, cabergoline, hydrocortisone | 341              | Alive with disease |
| 4 (1990)                 | 33/M    | 2                        | IA1   | Lobectomy, N1+N2                         | Typical         | Recurrent, ipsilateral lung | 111              | Thoracotomy, wedge resection, nodal dissection, mifepristone | 112              | Alive, tumor free |
| 5 (1998)                 | 20/F    | 1                        | IIIA  | Lobectomy, N1+N2                         | Atypical        | Recurrent, ipsilateral hilum, metastatic to liver | 55               | Thoracotomy, nodal dissection, bilateral adrenalectomy, hydrocortisone, fludrocortisone | 175              | Alive with disease |
| 6 (2001)                 | 39/M    | 1                        | IIIA  | Lobectomy, N1+N2                         | Typical         | Recurrent, ipsilateral mediastinum | 18                | Thoracotomy, nodal dissection, mifepristone, repeat VATS nodal dissection, bilateral adrenalectomy | 187              | Alive with disease |
| 7 (2007)                 | 22/F    | 1                        | IIIA  | Segment, N1+N2                           | Typical         | Recurrent, ipsilateral lung | 36                | Thoracotomy, lobectomy, lymph node dissection, bilateral adrenalectomy | 143              | Alive with disease |

\textit{M} = \text{Male}, \textit{F} = \text{Female}, \textit{UFC} = \text{urine free cortisol}, \textit{RT} = \text{radiotherapy}, \textit{VATS} = \text{Video Assisted Thoracoscopic Surgery}
Partial lymphadenectomy (N1 only), and stage based off of partial nodal staging
**Table 5: Characteristics of Patients with Persistent Disease**

| Patient (year of surgery) | Age/Sex | Time to Surgery (months) | Stage | Index Surgery, Extent of nodal dissection | Typical/Atypical | Persistent | Time to Recurrence (months) | Additional Therapy | Follow-up Months | Status at Last Follow-up |
|---------------------------|---------|--------------------------|-------|------------------------------------------|------------------|------------|----------------------------|------------------|-----------------|--------------------------|
| 1 (1983)                  | 28/F    | 4                        | T1aNX | Wedge                                    | Typical          | Persistent elevation in ACTH and UFC | NA              | Bilateral adrenalectomy   | 2                | Dead with persistent disease |
| 2 (1988)                  | 55/F    | 7                        | IA2   | Lobectomy, N1+N2                          | Typical          | Persistent elevation in ACTH and UFC | NA              | Two redo-thoracotomies, bilateral adrenalectomy | 117              | Dead with cancer |
| 3 (2017)                  | 80/F    | 1                        | IA2   | Wedge, N1+N2                              | Typical          | Persistent, contralateral lung and mediastinum | NA              | VATS lung wedge resection, nodal dissection | 26               | Alive, tumor free |
| 4 (2020)                  | 51/M    | 4                        | IA2   | Lobectomy, N1+N2                          | Typical          | Persistent elevation in ACTH           | NA              |                                | 1                | Alive with disease |

*M = Male, F = Female, UFC = urine free cortisol, RT = radiotherapy, NX = Nodes not sampled, VATS = Video Assisted Thoracosopic Surgery*
Supplemental Figure 1: Approach to the Diagnosis of Cushing’s Syndrome Secondary to Ectopic ACTH Secretion

* UFC and same day bedtime salivary cortisol weekly for 6 weeks

UFC = Urine Free Cortisol, DST = Dexamethasone Suppression Test, ACTH = Adrenocorticotropic hormone, MRI = Magnetic Resonance Imaging, IPSS = Inferior Petrosal Sinus Sampling, CRH = Corticotropin-Releasing Hormone, CT = Computed Tomography, PET = Positron Emission Tomography, $^{18}$F-DOPA = 18-Fluorodopa, $^{68}$Ga-DOTATATE = Gallium 68-DOTATATE, $^{18}$FDG = 18-Fluorodeoxyglucose, ADX = adrenalectomy

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