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ORIGINAL ARTICLE

# Insulinoma-associated protein 1 is a sensitive and specific marker for lung neuroendocrine tumors in cytologic and surgical specimens

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**KEYWORDS**
Insulinoma-associated protein 1;
INSM1;
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Neuroendocrine

**Introduction** Insulinoma-associated protein 1 (INSM1) is an immunohistochemical marker for neuroendocrine differentiation with potentially superior sensitivity and specificity. INSM1 performance in pulmonary cytology cell block material (CB) has not been well established, and large series demonstrating its performance have been few.

**Materials and methods** Typical and atypical carcinoid, small cell lung carcinoma, and large cell neuroendocrine carcinoma, squamous cell carcinoma, and adenocarcinoma CBs and 563 surgical specimens comprising 17 typical carcinoid, 14 atypical carcinoid, 8 small cell lung carcinoma, 10 large cell neuroendocrine carcinoma, 58 squamous cell carcinoma, 415 adenocarcinoma, and 17 large cell carcinoma cases and 24 other tumor types were immunostained with INSM1, CD56, synaptophysin, and chromogranin A.

**Results** The INSM1 sensitivity, specificity, positive predictive value, and negative predictive value were 92.3%, 100%, 78.9%, and 99% in the CBs and 89.8%, 98.1%, 81.5%, and 99% in the surgical specimens, respectively, with 86.2% concordance. The sensitivity, specificity, positive predictive value, and negative predictive value for the other neuroendocrine markers were 97.4%, 93.3%, 97.4%, and 93.3% in the CBs and 93.9%, 93.6%, 58.2%, and 99.4% in the surgical specimens for CD56; 89.7%, 100%, 100%, and 75% in the CBs and 93.4%, 91.2%, 50.5%, and 99.4% in the surgical specimens for synaptophysin; 66.7%, 100%, 100%, and 53.6% in the CBs and 75.5%, 98.6%, 84.1%, and 97.7% in the surgical specimens for chromogranin A, respectively. Finally, INSM1, together with CD56, maximized the sensitivity to 100% with 93.3% specificity in the CBs.

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**Conclusions** The results from our study have further established the high sensitivity and specificity of INSM1 in the largest pulmonary cytologic and surgical cohorts to date. INSM1 either matched or outperformed the performance of existing neuroendocrine markers, and its combination with CD56 appeared to maximize test performance.


## Introduction

Lung cancer is the third most common cancer in incidence and the most common cause of death from cancer in the United States.[1,2] Fine needle aspiration represents the mainstay diagnostic approach in the initial workup of a lung mass.[3] In particular, establishing the presence of neuroendocrine differentiation is crucial because the findings can alter the downstream clinical or surgical management.[4,5] Neuroendocrine tumors, such as small cell carcinoma, are more likely to be treated with chemotherapy, but non-neuroendocrine tumors, such as lung adenocarcinoma and squamous cell carcinoma, might be amenable to surgical excision. At present, a panel of CD56, synaptophysin (Syn), and chromogranin A (CGA) immunohistochemical markers is used to establish neuroendocrine differentiation in primary pulmonary tumors. However, the low sensitivity of CGA and low specificity of Syn and CD56 have made diagnostic interpretation challenging. Second, nonspecific background cytoplasmic and membranous marker staining, especially around areas of necrosis, can occur and can be difficult to interpret, especially in cases with focal or weak staining. Finally, 10% to 25% of high-grade histologic neuroendocrine carcinomas might not stain with a combination of all 3 neuroendocrine markers.[5-7] Thus, the canonical neuroendocrine markers, although valuable, still have significant drawbacks.

First isolated in 1992 from human insulinoma tissue, insulinoma-associated protein 1 (INSM1) is a 510-amino acid transcription factor with 5 zinc finger DNA-binding motifs.[8] INSM1 expression is spatially and temporally restricted to the central nervous system and neuroendocrine organs during development and is a key regulator of the neuroendocrine and neural differentiation programs in the central nervous system and other endocrine cells within other organs, including the pancreas, thyroid, pituitary, adrenal gland, kidney, stomach, spleen, testis, lung, liver, and colon.[8,9] Initial studies described INSM1 RNA overexpression specifically in tumors of neuroendocrine origin, including pheochromocytoma, insulinoma, medullary thyroid carcinoma, small cell lung carcinoma (SCLC), and carcinoid tumors.[9] During the past 2 decades, INSM1 has been increasingly recognized as a valuable sensitive and specific immunohistochemical marker for demonstrating the neuroendocrine properties in multiple organ systems and in tumors with neuroendocrine features.[10-21]

Recently, a shift occurred toward using material acquired through minimally invasive fine needle aspiration cytology and small biopsy specimens of pulmonary tumors for further diagnostic characterization and molecular studies.[3] Hence, a sensitive and specific marker that can be used to determine neuroendocrine differentiation to appropriately triage the management without exhausting the cellular material is crucial. In the context of pulmonary tumors, Rooper et al[18] demonstrated that INSM1 has superior sensitivity and specificity compared with CGA, Syn, and CD56 in surgical resection specimens and small biopsy samples (103 pulmonary neuroendocrine and 156 non-neuroendocrine cases). However, little is known regarding the performance of INSM1 in the context of lung cytology. Moreover, Rooper et al[18] had focused primarily on the INSM1 staining patterns in a limited number of common primary pulmonary tumor types (23 typical carcinoid [TC], 18 atypical carcinoid [AC], 39 SCLC, 23 large cell neuroendocrine carcinoma [LCNEC], 61 squamous cell carcinoma [SCC] and 95 adenocarcinoma [ADC] cases). The specificity of INSM1 among other non−SCLC types is not yet well established. In the present study, to address both these questions, we evaluated the diagnostic utility of INSM1 compared with CD56, Syn, and CGA in a cohort of cytology cell blocks (CBs) and the largest, most diverse cohort of surgical specimens to date.

## Materials and methods

The institutional review board at Weill Cornell Medicine reviewed and approved the present retrospective human tissue study.

CBs of TC (11 cases), AC (11 cases), SCLC (9 cases), LCNEC (8 cases), SCC (9 cases), and ADC (6 cases) from 2007 to 2018 were retrieved. In the preparation of these CBs, fine needle aspiration specimens were collected in 10% neutral buffered formalin. The CBs were then prepared by centrifuging for 7 minutes. The supernatant was discarded, and the resultant centrifuged material underwent routine processing and paraffin embedding as a CB and then staining with hematoxylin and eosin. In addition, whole slides were prepared from 32 formalin-fixed paraffin-embedded surgical resection specimens that included 8 TC, 9 AC, 5 SCLC, 2 SCC, and 2 ADC cases. Among these 32 surgical cases, 29 (8 TC, 8 AC, 5 SCLC, 6 LCNEC, 2 SCC, and 1 ADC) had matching corresponding CBs, which were used to establish concordance. With the inclusion of tumor microarrays (TMAs), a total of 17 TC, 14 AC, 13 SCLC, 10 LCNEC, 58 SCC, 415 ADC, 17 large cell carcinoma (LCC), and 24 other pulmonary tumor types (sarcomatoid carcinoma, 11 cases; adenosquamous carcinoma, 10 cases; inflammatory

myofibroblastic tumor, 1 case; and solitary fibrous tumor, 2 cases) were analyzed to establish the specificity for the surgical specimens. The TMA slides were created in-house with tumor cores either in duplicate, with each core measuring 1.5 mm, or in triplicate, with each core measuring 1.0 mm. All the tumor specimens used in the present study were reviewed by the original pathologist and a second thoracic pathologist and then de-identified as to their source.

Immunohistochemical staining (IHC) was performed using INSM1 antibody (1:100; catalog no., sc-271408; Santa Cruz Biotechnology, Inc., Dallas, TX), CD56 (ready-to-use; catalog no., PA0191; clone CD564; Leica Biosystems, Buffalo Grove, IL), Syn (ready-to-use; catalog no., PA0299; clone 27G12; Leica Biosystems), and CGA (1:400; catalog no., MU126-UC; clone LK2H10; Biogenex, Fremont, CA) on paraffin-embedded tissue sections using a Leica Bond system (Leica Biosystems) using the modified protocol F provided by the manufacturer. The section was pretreated using heat-mediated antigen retrieval with sodium citrate buffer (pH, 6; epitope retrieval solution 1) for 30 minutes and incubated with the antibodies for 60 minutes at room temperature. All 4 markers were detected using a horseradish peroxidase-conjugated compact polymer system and 3,3' diaminobenzidine as the chromogen. Each section was counterstained with hematoxylin and mounted with Leica Micromount (Leica Biosystems). INSM1, CD56, Syn, and CGA IHC were graded as 1+, 2+, or 3+. For the CBs, 2+ and 3+ were considered positive; for the surgical specimens, $\geq$1+ in >5% of cells was considered positive. In general, positive cases were diffuse, with whole slide cases providing guidance regarding the confidence in TMA staining.

To determine the optimal combination of neuroendocrine markers for IHC in cytology, we compared the IHC data among the 4 neuroendocrine markers in various permutations, with each cytology case considered positive if IHC of any of the neuroendocrine markers in the combination was positive. The sensitivity and specificity were then calculated for each combination.

## Results

Cytology CBs, prepared from fine needle aspirates from 54 neuroendocrine and non-neuroendocrine pulmonary tumors, and 563 surgical resection and TMA specimens were examined. The average patient age within the cytology cohort was 68 years (range, 32-86 years) with a 1.3:1 male/female ratio and a mean tumor size of 3 cm (range, 0.7-9.5

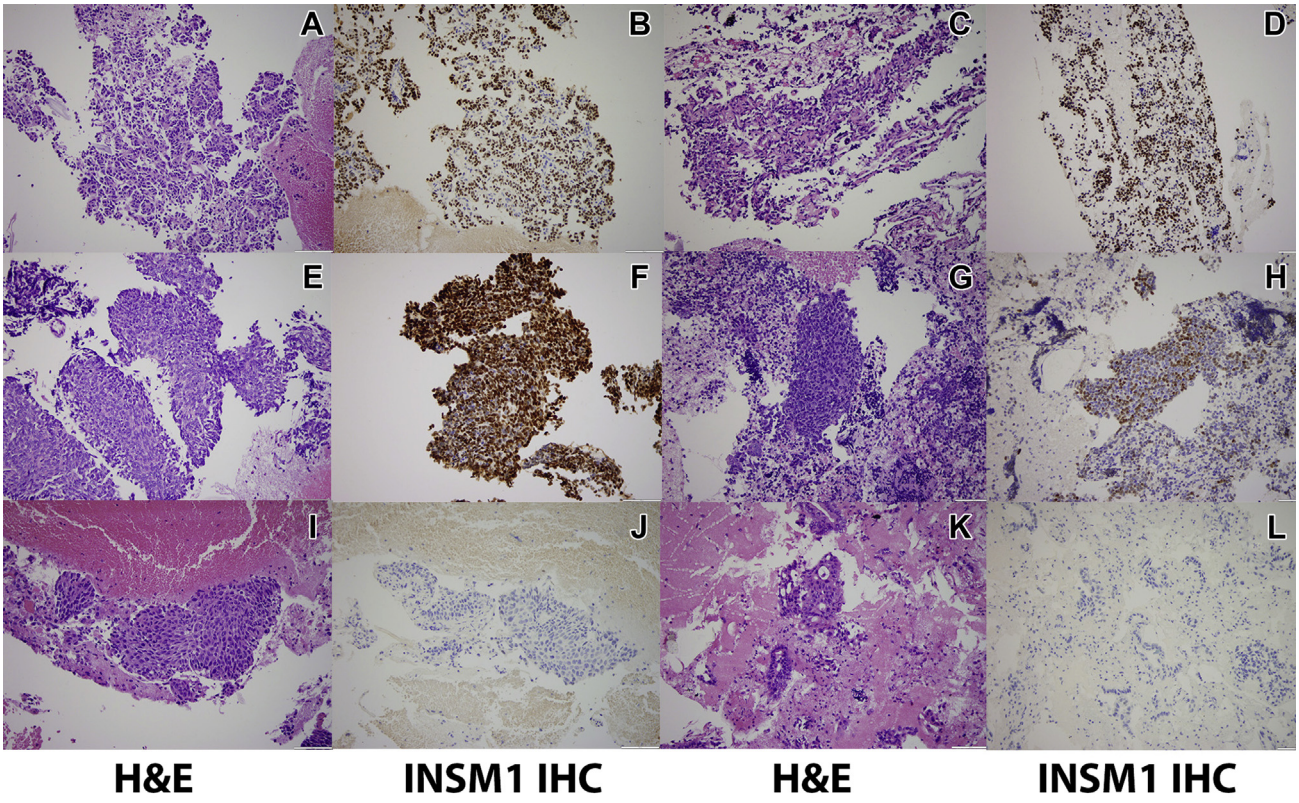


**Figure 1** Insulinoma-associated protein 1 (INSM1) immunohistochemistry (IHC) in cytologic specimens. Cell block preparations from fine needle aspirates from A, B, typical carcinoid, C, D, atypical carcinoid, E, F, small cell lung cancer, G, H, large cell neuroendocrine carcinoma, I, J, squamous cell carcinoma, and K, L, adenocarcinoma were stained with either hematoxylin and eosin (H&E) or immunostained with INSM1. Nuclear INSM1 was seen primarily in pulmonary neuroendocrine tumors and not in non-neuroendocrine malignancies in the cytologic specimens. All images were captured at 200× magnification.

cm). All cases were tested in the primary tumor, except for 6 cases of AC, 3 cases of SCLC, and 2 cases of LCNEC, which were secondary metastases of pulmonary origin to either lymph nodes or the liver.

Among the CB preparations, 100% of the TC (11 of 11), 100% of the AC (11 of 11), 89% of the SCLC (8 of 9), and 75% of the LCNEC (6 of 8) cases showed strong INSM1 staining. No significant differences in the degree of INSM1 staining were noted among the neuroendocrine tumors in the cytology specimens. In addition, in the non-neuroendocrine cytology specimens, the CB preparations from pulmonary SCC and ADC did not demonstrate any nuclear INSM1 staining (Fig. 1). In the cytology specimens, INSM1 staining showed a sensitivity of 92.3% and specificity of 100% for pulmonary neuroendocrine tumors.

Similar to the cytologic specimens, after combining the pulmonary neuroendocrine cases from the surgical resection specimens and TMA, INSM1 was present in 94.1% of TC (16 of 17) and 85.7% of AC (12 of 14), 100% of SCLC (8 of 8), and 80% of LCNEC (8 of 10) cases. In contrast, with respect to the pulmonary non-neuroendocrine tumors in both the matched resection specimens and the TMAs, INSM1 was seen in 1.7% of SCC (1 of 58), 1.2% of ADC (5 of 415), 17.6% of LCC (3 of 17), and 4.2% of other pulmonary tumors (1 of 24; Fig. 2). Overall, 10 of 511 non−small cell lung cancer (NSCLC) tumors (2.0%), after exclusion of the 2 solitary fibrous tumors and 1 inflammatory myofibroblastic tumor, demonstrated $\geq$1+ INSM1 positivity in >5% of the tumor. Four cases of ADC showed focal 1+ INSM1 expression with <5% staining but did not meet the threshold to be considered positive. Among the 24 other pulmonary tumor types, only a single case of sarcomatoid carcinoma showed 2+ INSM1 expression. Taken together, INSM1 showed an 89.8% sensitivity and 98.1% specificity for pulmonary neuroendocrine tumors in the surgical and TMA specimens.

Among the 29 cases with cytology−histology correlation, concordance was seen in 25 (86.2%). All 4 discordant cases (1 of SCLC, 2 of LCNEC, and 1 of SCC) were negative in cytology but demonstrated INSM1 positivity in the surgical specimens. Overall, INSM1 expression showed 89.8% to 92.3% sensitivity and 98.1% to 100% specificity for pulmonary neuroendocrine tumors in the cytology and surgical specimens. The positive predictive value (PPV) and negative predictive value (NPV) for INSM1 IHC for pulmonary neuroendocrine tumors was 100% and 78.9% on cytology and 81.5% and 99% on the surgical specimens, respectively (Table 1).

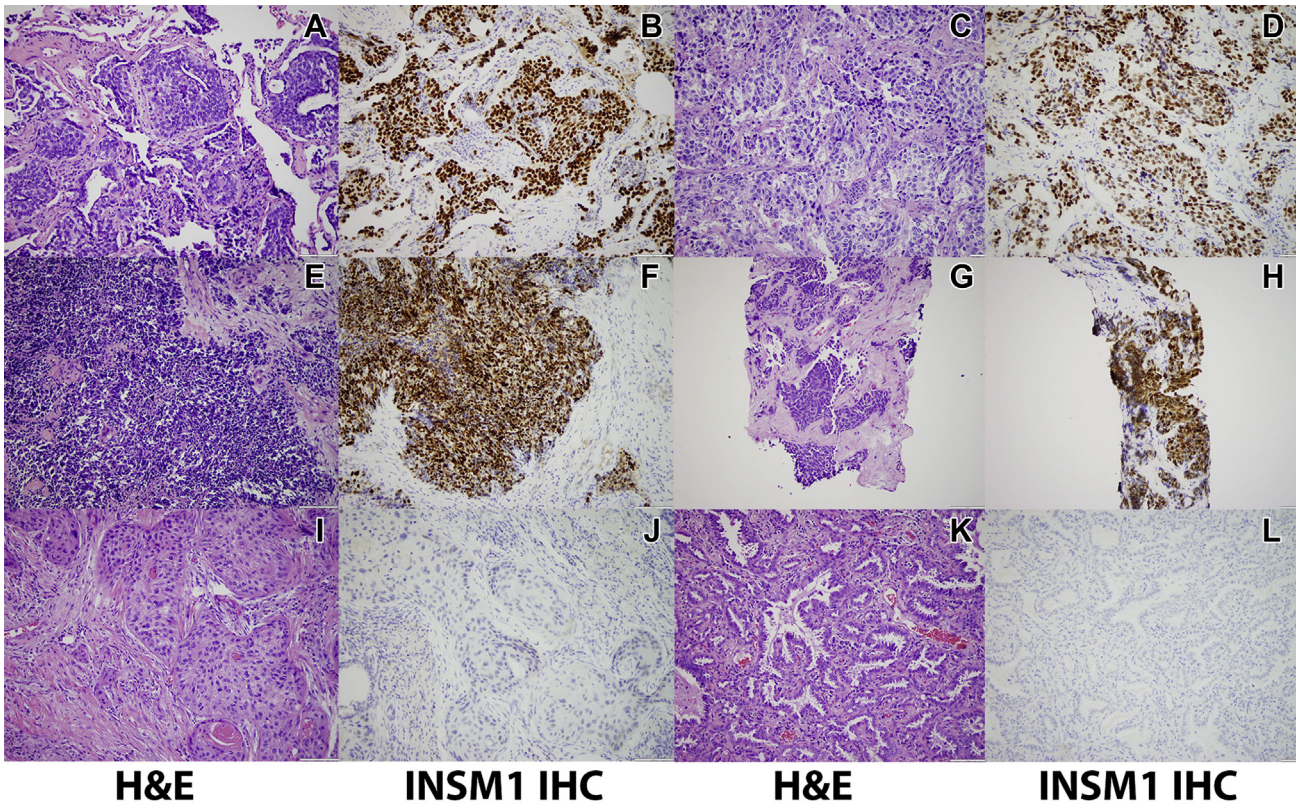


**Figure 2** Insulinoma-associated protein 1 (INSM1) immunohistochemistry (IHC) in surgical specimens. Representative sections from surgical resection specimens of A, B, typical carcinoid, C, D, atypical carcinoid, E, F, small cell lung cancer, G, H, large cell neuroendocrine carcinoma, I, J, squamous cell carcinoma, and K, L adenocarcinoma were stained with either hematoxylin and eosin (H&E) or immunostained with INSM1. Similar to cytologic specimens, INSM1 was seen primarily in typical and atypical carcinoid specimens. All images were captured at 200× magnification.

**Table 1** Overall test parameters for INSM1 compared with CD56, synaptophysin, and chromogranin A.

| Marker | TP (n) | FP (n) | TN (n) | FN (n) | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|---|---|---|
| Cytology | | | | | | | | |
| INSM1 | 36 | 0 | 15 | 3 | 92.3 | 100 | 100 | 78.9 |
| CD56 | 38 | 1 | 14 | 1 | 97.4 | 93.3 | 97.4 | 93.3 |
| Syn | 34 | 0 | 15 | 5 | 89.7 | 100 | 100 | 75 |
| CGA | 26 | 0 | 15 | 13 | 66.7 | 100 | 100 | 53.6 |
| Surgical and TMA specimens | | | | | | | | |
| INSM1 | 44 | 10 | 504 | 5 | 89.8 | 98.1 | 81.5 | 99 |
| CD56 | 46 | 33 | 481 | 3 | 93.9 | 93.6 | 58.2 | 99.4 |
| Syn | 46 | 45 | 469 | 3 | 93.4 | 91.2 | 50.5 | 99.4 |
| CGA | 37 | 7 | 507 | 12 | 75.5 | 98.6 | 84.1 | 97.7 |

Abbreviations: CGA, chromogranin A; FN, false-negative INSM1 staining in neuroendocrine tumors; FP, false-positive INSM1 staining in non-neuroendocrine tumors; INSM1, insulinoma-associated protein 1; NPV, negative predictive value; PPV, positive predictive value; Syn, synaptophysin; TMA, tissue microarray; TN, true-negative INSM1 staining in non-neuroendocrine tumors; TP, true-positive INSM1 staining in neuroendocrine tumors.

In contrast, CD56 expression was seen in 100% of TC (11 of 11), 91% of AC (10 of 11), 100% of SCLC (9 of 9), 100% of LCNEC (8 of 8), 11% of SCC (1 of 9), and 0% of ADC (0 of 6) cases in the CBs. CD56 expression was seen in 100% of TC (17 of 17), 85.7% of AC (12 of 14), 100% of SCLC (8 of 8), 90% of LCNEC (9 of 10), 5.2% of SCC (3 of 58), 4.8% of ADC (20 of 415), and 17.6% of LCC (3 of 17) cases and 29.2% of other pulmonary tumor subtypes (7 of 24) in the surgical specimens. Syn expression was seen in 100% of the TC (11 of 11), 100% of AC (11 of 11), 78% of SCLC (7 of 9), 63% of LCNEC (5 of 8), 0% of SCC (0 of 9), and 0% of ADC (0 of 6) cases in the CBs. Syn expression was seen in 100% of TC (17 of 17), 92.9% of AC (13 of 14), 100% of SCLC (8 of 8), 80% of LCNEC (8 of 10), 8.6% of SCC (5 of 58), 8.7% of ADC (36 of 415), and 11.8% of LCC (2 of 17) cases and 8.3% of other pulmonary tumor subtypes (2 of 24) in the surgical specimens. CGA expression was seen in 100% of TC (11 of 11), 100% of AC (11 of 11), 22% of SCLC (2 of 9), 25% of LCNEC (2 of 8), 0% of SCC (0 of 9), and 0% of ADC (0 of 6) cases in the CBs. CGA expression was seen in 100% of TC (17 of 17), 92.9% of AC (13 of 14), 37.5% of SCLC (3 of 8), 40% of LCNEC (4 of 10), 1.7% of SCC (1 of 58), 1.2% of ADC (5 of 415), and 5.9% of LCC (1 of 17) cases and 0% of other pulmonary tumor subtypes (0 of 24) in the surgical specimens. Among the LCC tumors, 35% (6 of 17) were positive for $\geq$1 neuroendocrine marker, with 1 tumor expressing all 4 neuroendocrine markers (Table 2).

Overall, the sensitivity, specificity, PPV, and NPV for CD56 were 97.4%, 93.3%, 97.4%, and 93.3% in the CBs and 93.9%, 93.6%, 58.2%, and 99.4% in the surgical specimens, respectively. The sensitivity, specificity, PPV, and NPV for Syn were 89.7%, 100%, 100%, and 75% in the CBs and 93.4%, 91.2%, 50.5%, and 99.4% in the surgical specimens, respectively. Finally, the sensitivity, specificity, PPV, and NPV for CGA were 66.7%, 100%, 100%, and 53.6% in the CBs and 75.5%, 98.6%, 84.1%, and 97.7% in the surgical specimens, respectively. The performance summary for INSM1 and all 3 neuroendocrine markers are summarized in Tables 1 and 3.

The IHC data were further examined to determine the effect of various neuroendocrine marker permutations on the sensitivity and specificity in the cytology specimens. The combination of INSM1 with CD56 demonstrated a sensitivity of 100% and a specificity of 93.3%, which was not further improved by the addition of the neuroendocrine markers. Certain high-grade neuroendocrine carcinoma cases could be definitively identified with the combination of INSM1 and CD56 (Fig. 3). In contrast, the addition of INSM1 with either Syn or CGA showed a sensitivity of 92.3% and 100% specificity (Table 4).

**Table 2** Characterization of large cell carcinoma cases with neuroendocrine differentiation.

| LCC case no. | INSM1 | CD56 | Syn | CGA |
|---|---|---|---|---|
| 1 | N | N | N | N |
| 2 | N | N | N | N |
| 3 | N | N | N | N |
| 4 | N | N | N | N |
| 5 | P | P | P | P |
| 6 | N | N | N | N |
| 7 | N | P | N | N |
| 8 | P | N | N | N |
| 9 | N | N | N | N |
| 10 | N | N | N | N |
| 11 | N | N | N | N |
| 12 | N | N | P | N |
| 13 | N | P | N | N |
| 14 | N | N | N | N |
| 15 | P | N | N | N |
| 16 | N | N | N | N |
| 17 | N | N | N | N |

Abbreviations: CGA, chromogranin A; INSM1, insulinoma-associated protein 1; LCC, large cell carcinoma; N, negative; P, positive; Syn, synaptophysin.

**Table 3** INSM1, CD56, synaptophysin, and chromogranin A performance summary in cytology and surgical specimens.

| Tumor type | INSM1 | CD56 | Synaptophysin | Chromogranin A |
|---|---|---|---|---|
| Cell block preparations | | | | |
| Typical carcinoid | 100 (11/11) | 100 (11/11) | 100 (11/11) | 100 (11/11) |
| Atypical carcinoid | 100 (11/11) | 91 (10/11) | 100 (11/11) | 100 (11/11) |
| Small cell lung carcinoma | 89 (8/9) | 100 (9/9) | 78 (7/9) | 22 (2/9) |
| Large cell neuroendocrine carcinoma | 75 (6/8) | 100 (8/8) | 63 (5/8) | 25 (2/8) |
| Squamous cell carcinoma | 0 (0/9) | 11 (1/9) | 0 (0/9) | 0 (0/9) |
| Adenocarcinoma | 0 (0/6) | 0 (0/6) | 0 (0/6) | 0 (0/6) |
| Surgical and TMA specimens | | | | |
| Typical carcinoid | 94.1 (16/17) | 100 (17/17) | 100 (17/17) | 100 (17/17) |
| Atypical carcinoid | 85.7 (12/14) | 85.7 (12/14) | 92.9 (13/14) | 92.9 (13/14) |
| Small cell lung carcinoma | 100 (8/8) | 100 (8/8) | 100 (8/8) | 37.5 (3/8) |
| Large cell neuroendocrine carcinoma | 80 (8/10) | 90 (9/10) | 80 (8/10) | 40 (4/10) |
| Squamous cell carcinoma | 1.7 (1/58) | 5.2 (3/58) | 8.6 (5/58) | 1.7 (1/58) |
| Adenocarcinoma | 1.2 (5/415) | 4.8 (20/415) | 8.7 (36/415) | 1.2 (5/415) |
| Large cell carcinoma | 17.6 (3/17) | 17.6 (3/17) | 11.8 (2/17) | 5.9 (1/17) |
| Other | 4.2 (1/24) | 29.2 (7/24) | 8.3 (2/24) | 0 (0/24) |

Abbreviations: INSM1, insulinoma-associated protein 1; TMA, tissue microarray.
Data presented as % (n/N).

## Discussion

The determination of neuroendocrine differentiation in biopsied or resected tumors can have significant effects on the subsequent clinical management.[2,4,5] This has been especially important given that neuroendocrine tumors are not uncommon, comprising ≤25% of primary pulmonary neoplasms.[22] The use of a combination of morphologic features and immunohistochemical markers has been recognized to improve the diagnostic sensitivity of SCLC and is considered a requirement by the World Health Organization in the diagnosis of LCNEC.[2,5,23] Most institutions use cytoplasmic CGA and Syn and/or membranous CD56 neuroendocrine immunohistochemical

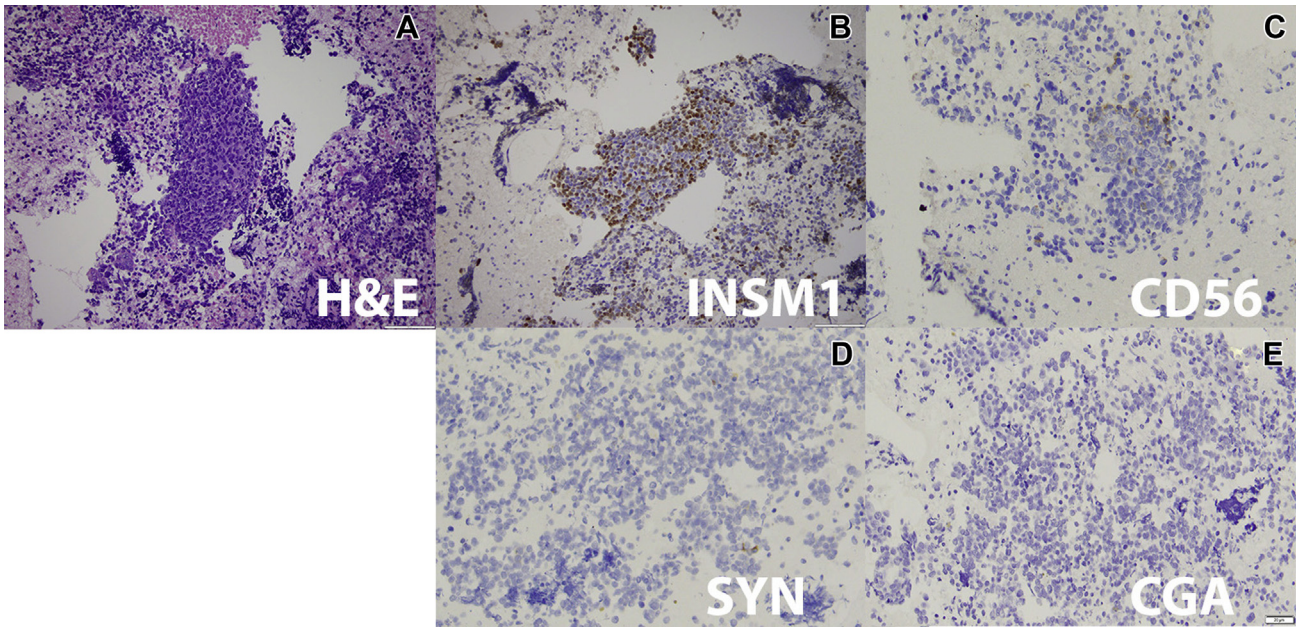


**Figure 3** A combination of insulinoma-associated protein 1 (INSM1) and CD56 was useful for identifying pulmonary neuroendocrine tumors in cytology specimens. An example of a cell block preparation of a large cell neuroendocrine carcinoma stained with either A, eosin or hematoxylin or immunostained with B, INSM1, C, CD56, D, synaptophysin, and E, chromogranin A. Although neither synaptophysin nor chromogranin A demonstrated any staining, INSM1 demonstrated positive staining in a large proportion of cells, outperforming CD56 in this case, which was more focal. All images were captured at 200× magnification.

**Table 4** Sensitivity and specificity of neuroendocrine marker combinations for pulmonary tumor cytology.

| Combination | TP (n) | FP (n) | TN (n) | FN (n) | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|---|
| INSM1 only | 36 | 0 | 15 | 3 | 92.3 | 100 |
| CD56 only | 38 | 1 | 14 | 1 | 97.4 | 93.3 |
| Syn only | 34 | 0 | 15 | 5 | 87.2 | 100 |
| CGA only | 26 | 0 | 15 | 13 | 66.7 | 100 |
| INSM1 and CD56 | 39 | 1 | 14 | 0 | 100 | 93.3 |
| INSM1 and Syn | 36 | 0 | 15 | 3 | 92.3 | 100 |
| INSM1 and CGA | 36 | 0 | 15 | 3 | 92.3 | 100 |
| INSM1, CD56, and Syn | 39 | 1 | 14 | 0 | 100 | 93.3 |
| INSM1, CD56, and CGA | 39 | 1 | 14 | 0 | 100 | 93.3 |
| INSM1, Syn, and CGA | 39 | 1 | 14 | 0 | 100 | 93.3 |
| INSM1, CD56, Syn, and CGA | 39 | 1 | 14 | 0 | 100 | 93.3 |

Abbreviations: CGA, chromogranin A; FN, false-negative INSM1 staining in neuroendocrine tumors; FP, false-positive INSM1 staining in non-neuroendocrine tumors; INSM1, insulinoma-associated protein 1; Syn, synaptophysin; TN, true-negative INSM1 staining in non-neuroendocrine tumors; TP, true-positive INSM1 staining in neuroendocrine tumors.

markers; however, none of these 3 markers have both high sensitivity and high specificity.[18] Furthermore, a combination of these markers can be negative in 10% to 25% of high-grade neuroendocrine tumors.[5-7] Similar challenges have also been noted in cytologic CB specimens.[24,25] Thus, a need exists to explore novel neuroendocrine markers that can demonstrate both high sensitivity and high specificity from limited material.

Recent work has highlighted INSM1 as a valuable marker for demonstrating the neuroendocrine properties of tumors within the lung and multiple other organ systems, with superior sensitivity and specificity reported.[10,11,13-15,17-21] However, all the previous studies had only evaluated small biopsy samples and surgical resection specimens. Thus, little has been known of the performance of INSM1 in cytologic specimens. To date, only 2 studies have examined the use of INSM1 in lung CB preparations. Doxtader and Mukhopadhyay[12] performed INSM1 immunostaining on 74 primary lung tumor specimens that included 52 neuroendocrine neoplasms and 22 non-neuroendocrine tumors. In their study, nuclear INSM1 was identified in 93% of SCLC cases, 90% of TC cases, and 1 LCNEC case, with an overall sensitivity of 92% for pulmonary neuroendocrine tumors and specificity of 100%.[12] Similarly, Rodriguez et al[16] examined 32 cytologic cases of SCLC and demonstrated that 84% demonstrated diffuse INSM1 staining and 13% showed focal or weak INSM1 expression, with an overall sensitivity of 97%. Although our cohort of SCLC cases was smaller than those in both studies, we were able to identify INSM1 expression in 100% of our SCLC cases, which was slightly greater sensitivity. Overall, the sensitivity and specificity of 90.5% and 100%, respectively, in our pulmonary cytologic specimens were in keeping with both previous studies.[12,16] However, in addition to our cohort having a larger number of LCNEC cases compared with Doxtader and Mukhopadhyay,[12] our study is the first, to the best of our knowledge, to show diffuse INSM1 expression in AC cases in CB material, which had not been previously tested. Thus, not only were our findings in line with previous work confirming that INSM1 has utility in identifying neuroendocrine differentiation in small cytology specimens, but we have also supplanted the current data with INSM1 staining in both TC and AC cases.

Our INSM1 IHC findings in the matched surgical specimens also showed concordance with our cytologic findings. All neuroendocrine tumors showed diffuse moderate to strong (2+ to 3+) INSM1 expression. INSM1 expression did not appear to be significantly different among the pulmonary neuroendocrine neoplasms; thus, distinguishing the subtypes in small specimens can be challenging. However, the morphologic features in conjunction with other markers, such as orthopedia homeobox protein, can aid in determining the definitive diagnosis of pulmonary carcinoid tumors, especially in small biopsy samples or CB material.[26]

In contrast, in our surgical resection specimens, 1 case of SCC and 1 case of ADC showed 1+ INSM1 staining in >5% of the tumor and were considered positive. With every immunohistochemical marker, the specificity for the target antigens is crucial. Nonspecific staining in tumors can lead to diagnostic misinterpretation and incorrect clinical or surgical management. To further characterize INSM1 specificity, we examined a large cohort of non-neuroendocrine tumors on TMAs that included common variants of ADC (solid, mucinous, papillary, micropapillary, lepidic), SCC, adenosquamous carcinoma, sarcomatoid carcinoma, and LCC, among others. In our cohort of lung NSCLC tumors (after exclusion of the 2 cases of solitary fibrous tumors and 1 case of an inflammatory myofibroblastic tumor), we found 2.7% of tumors (14 of 511) demonstrated weak 1+ to moderate 2+ staining, including tumors with <5% staining that were not considered positive (Fig. 4A). The non-neuroendocrine NSCLC tumors that showed any INSM1 staining included 1% of ADC (5 of 511), 0.2% of sarcomatoid carcinoma (1 of 511), 0.2% of SCC (1 of 511), and 0.6% of LCC (3 of 511) cases.

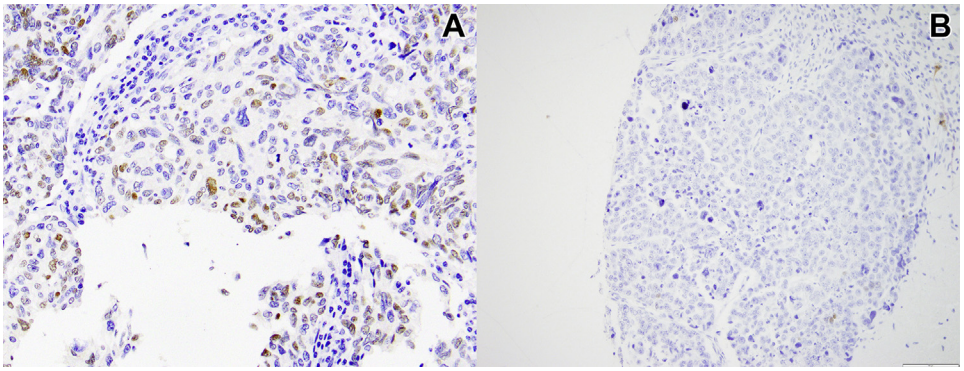


**Figure 4** False-positive insulinoma-associated protein 1 (INSM1) staining in non-neuroendocrine tumors and false-negative INSM1 staining in neuroendocrine tumors. Tumor microarrays with both non-neuroendocrine and neuroendocrine tumors were immunostained with INSM1. Representative examples of a A, false-positive non-neuroendocrine case and B, false-negative neuroendocrine tumor shown. All images were captured at 200× magnification.

Another important caveat to consider with immunohistochemical markers is that intratumoral variability of staining can occur, and the interpretation can be affected, depending on the regions sampled. However, in our study, our true INSM1-positive cases were diffuse across tumors on the whole slide resection specimens, CBs, and TMAs. In addition, TMAs were created with tumor cores in duplicate or triplicate and, thus, provided a better overall representation of the tumor. Therefore, the diffuse INSM1 staining observed on the CB and resection specimen whole slides provided us with confidence regarding the INSM1 staining seen on the TMAs.

The LCC group of tumors within our cohort (n = 17) were historically characterized as such based solely on their morphology without the use of immunostaining. Recent ongoing discussions in reported studies have proposed the stratification of NSCLCs (including LCC) into 2 distinct categories—those without morphologic neuroendocrine features but with immunohistochemical evidence of ≥1 neuroendocrine marker, and those without either morphologic or immunohistochemical neuroendocrine features (in this case, NSCLC, not otherwise specified).[27] In our cohort, 35% (6 of 17) showed positivity with ≥1 neuroendocrine marker and would fit the criteria for LCC-neuroendocrine. Because we did not substratify our LCC cases into the different categories in the present study, the inclusion of LCC-neuroendocrine cases into our main LCC cohort would have falsely decreased the specificity of INSM1. In contrast, it raises the possibility that in certain cases of LCNEC that demonstrate the appropriate morphologic neuroendocrine features but not neuroendocrine marker staining might be falsely characterized as LCC, which could also affect the sensitivity and specificity. In our cohort, 1 case of LCC was positive for all 4 neuroendocrine markers, which raises the possibility that this tumor might have been a misclassified LCNEC that did not fit the morphologic criteria at diagnosis.

Similarly, the faint INSM1 staining in other NSCLC subtypes could either reflect focal neuroendocrine differentiation within the tumor or could be nonspecific and related to the staining protocol. Cases of non−SCLC with neuroendocrine differentiation have been reported.[28-31] Ionescu et al[28] demonstrated that in their cohort of 558 NSCLC tumors, 13.6% showed ≥1% staining with CD56, Syn, or CGA. However, all the studies showed that the presence of neuroendocrine marker positivity had little effect on the clinical prognostic significance.[28-31] Thus, the prognostic significance for the detection of neuroendocrine markers in tumors that do not meet the morphologic criteria remains unclear. The current recommendation is not to use neuroendocrine immunohistochemical markers for NSCLC unless distinct morphologic neuroendocrine features are present.[27,32]

In contrast, INSM1 IHC has not shown 100% sensitivity in all studies of pulmonary tumors to date. Rooper et al[18] found 5% of SCLC and 9% of LCNEC cases were negative for INSM1. In the study by Doxtader and Mukhopadhyay,[12] 8% of SCLC and 10% of TC cases were negative for INSM1. Similarly, 3% of SCLC cases were negative in the study by Rodriguez et al.[16] Even within our own cohort, 7.7% of neuroendocrine tumors, which showed immunoreactivity with CD56, Syn, or CGA, did not show any INSM1 staining in cytology and in only 10.2% of surgical resections (Fig. 4B). Also, we did not identify any cases in our cohort that were positive for INSM1 but negative for all of the other neuroendocrine markers. The differences in fixation conditions, age of the TMAs, and staining protocol in our study could have affected our INSM1 IHC findings. INSM1 expression can also be affected by the treatment effect.[16] Thus, optimization of the threshold for considering a tumor INSM1 positive will likely depend on institution-dependent staining protocols and the level of experience of the reviewing pathologist. Nonetheless, caution is necessary to avoid relying solely on

the INSM1 staining pattern. It should be considered in the context of the cell morphology and radiologic features when assessing small specimens. Regardless, in both scenarios, diagnostic interpretation should not rely exclusively on INSM1 but should be in conjunction with the morphology to avoid the pitfalls, especially with cytology specimens, with the caveat that positive INSM1 findings will be more meaningful (PPV, 100%) than negative ones (NPV, 78.9%).

Within our cohort of CBs and surgical specimens, INSM1 demonstrated 89.8% to 92.3% sensitivity and 98.1% to 100% specificity, with a PPV and NPV of 81.5% to 100% and 78.9% to 99%, respectively. The sensitivity of INSM1 was greater than that of CGA (66.7%-75.5%) and comparable to that of CD56 (93.9%-97.9%) and Syn (89.7%-93.4%). The specificity of INSM1 was greater than the specificity of CD56 (93.3%-93.6%) and comparable to the specificity of Syn (91.2%-100%) and CGA (98.6%-100%; Table 1). Multiple studies have explored the sensitivity and specificity of CD56, Syn, and CGA in neuroendocrine and non-neuroendocrine tumors in cytologic and surgical specimens.[12,14,16,17,19] Doxtader and Mukhopadhyay[12] reported that INSM1 had a slightly lower or comparable sensitivity but greater specificity compared with any of the 3 traditional neuroendocrine markers (92% versus 100% sensitivity, 100% versus 94% specificity). Rodriguez et al[16] found that INSM1 had a sensitivity of 97% in their cohort of SCLC cases, slightly greater than the 96% sensitivity seen with CD56. In surgical specimens, Fujino et al[14] demonstrated that INSM1 had a superior sensitivity of 98% compared with 82.3% for CGA, 86.2% for Syn, and 79.4% for CD56. Similar findings were also recapitulated by Rooper et al,[18] who reported 96.4% overall sensitivity for INSM1 compared 87.4% for any of the 3 traditional markers. Finally, a recent study by Mukhopadhyay et al[33] demonstrated a sensitivity of 95% for INSM1, which was greater than that for CGA (84%) and similar to that for Syn (98%) and CD56 (97%). The specificity of INSM1 in their study was also 97%, greater than that for Syn (90%) and CD56 (87%) and similar to that for CGA (98%).[33] Thus, consistent with other studies reported to date, the overwhelming evidence has demonstrated that INSM1 has very high sensitivity, similar to that of CD56 and Syn, with a matching high specificity, similar to that for CGA.

To the best of our knowledge, our study is also the first to explore the optimal combination of neuroendocrine markers for IHC on cytology specimens from neuroendocrine pulmonary tumors. Compared with INSM1 alone, the addition of CD56 increased the sensitivity of detecting pulmonary neuroendocrine tumors in cytology to 100%. However, the specificity was offset by a single case of SCC that demonstrated positivity for CD56 but was negative for CGA and Syn. The addition of CGA and Syn to the combination of INSM1 and CD56 did not further improve the sensitivity or specificity. The reduction in specificity with the combination was not surprising, given the known lower specificity of CD56 alone compared with the other neuroendocrine markers, Syn and CGA.[12,14,16,17,19] However, compared with other combinations (ie, INSM1 with Syn or INSM1 with CGA), we would recommend the combination of INSM1 and CD56, given the maximal detection of pulmonary neuroendocrine tumors on cytology without significantly sacrificing specificity in double-positive cases. The addition of CGA or Syn in INSM1-negative, CD56-positive cases is an interesting question for future study. Furthermore, as alluded to previously, the morphology of the neoplastic population will provide useful guidance when ordering specific immunohistochemical stains.

## Conclusions

Our data have demonstrated that INSM1 is the first neuroendocrine marker with both high sensitivity and high specificity for primary pulmonary neuroendocrine tumors in cytologic and surgical specimens. Although additional studies are necessary to determine whether INSM1 can replace the current trio of neuroendocrine markers (ie, Syn, CGA, CD56), it can serve as a useful adjunct in cases in which extensive necrosis could lead to nonspecific background staining with the canonical markers or when the specimen is significantly limiting. INSM1 cross-reactivity with other NSCLC types could be observed in a small subset of cases. In contrast, a small subset of neuroendocrine tumors might not stain with INSM1 in either cytologic or surgical specimens. Thus, although INSM1 is a useful marker for the determination of neuroendocrine differentiation, the immunostaining findings should be considered in conjunction with other morphologic parameters when determining the final diagnosis to avoid misinterpretation.

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