Diagnostic Value of Insulinoma-Associated Protein 1 (INSM1) and Comparison With Established Neuroendocrine Markers in Pulmonary Cancers

A Comprehensive Study and Review of the Literature

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- **Context.**—The diagnostic distinction of pulmonary neuroendocrine (NE) tumors from non–small cell lung carcinomas (NSCLCs) is clinically relevant for prognostication and treatment. Diagnosis is based on morphology and immunohistochemical staining.

- **Objective.**—To determine the diagnostic value of insulinoma-associated protein 1 (INSM1), in comparison with established NE markers, in pulmonary tumors.

- **Design.**—Fifty-four pulmonary NE tumors and 632 NSCLCs were stained for INSM1, CD56, chromogranin A, and synaptophysin. In a subset, gene expression data were available for analysis. Also, 419 metastases to the lungs were stained for INSM1. A literature search identified 39 additional studies with data on NE markers in lung cancers from the last 15 years. Seven of these included data on INSM1.

- **Results.**—A positive INSM1 staining was seen in 39 of 54 NE tumors (72%) and 6 of 623 NSCLCs (1%). The corresponding numbers were 47 of 54 (87%) and 14 of 626 (2%) for CD56, 30 of 54 (56%) and 6 of 629 (1%) for chromogranin A, and 46 of 54 (85%) and 49 of 630 (8%) for synaptophysin, respectively. Analysis of literature data revealed that CD56 and INSM1 were the best markers for identification of high-grade NE pulmonary tumors when considering both sensitivity and specificity, while synaptophysin also showed good sensitivity. INSM1 gene expression was clearly associated with NE histology.

- **Conclusions.**—The solid data of both our and previous studies confirm the diagnostic value of INSM1 as a NE marker in pulmonary pathology. The combination of CD56 with INSM1 and/or synaptophysin should be the first-hand choice to confirm pulmonary high-grade NE tumors. INSM1 gene expression could be used to predict NE tumor histology.


Primary pulmonary neuroendocrine (NE) tumors include the high-grade tumors small cell lung carcinoma (SCLC) and large cell neuroendocrine carcinoma (LCNEC) and the less aggressive carcinoid tumors (CTs), further divided into typical and atypical CT. Treatment differs from that of non–small cell lung carcinomas (NSCLCs), mainly consisting of adenocarcinomas (ACs) and squamous cell carcinomas (SqCCs), with respect to choice of chemotherapy regimens and the extensive use of immunomodulating and targeted therapies in advanced-stage NSCLC.

Apart from NE morphology (eg, organoid nests, trabecular growth, rosette-like structures, peripheral palisading pattern), an LCNEC diagnosis requires at least 1 positive immunohistochemical (IHC) NE marker. A positive NE marker is not strictly required for SCLC, though most cases do express at least 1 such marker. Carcinoid tumors are typically positive for all, or at least several, NE markers. Neuroendocrine markers may also be expressed in NSCLC, which may cause diagnostic problems, and staining with NE markers is only recommended if NE morphology is evident.
In the current World Health Organization (WHO) classification, synaptophysin, chromogranin A, and CD56 are recommended as NE markers with synaptophysin and chromogranin A suggested as the first-hand choice. In the recent best practices recommendation from the WHO group, a panel of all 3 markers is recommended for identification of NE tumors (though the lack of consensus concerning number of markers is pointed out), and any positivity is suggested to define a positive staining instead of at least 10% of the cells as recommended in the WHO classification.

Synaptophysin and CD56 are known to be more sensitive but less specific markers, while chromogranin A is very specific but has limited sensitivity. Insulinoma-associated protein 1 (INSM1) is a new NE marker that has been investigated in pulmonary tumors in recent studies. INSM1 is suggested to have a rather good sensitivity and a very good specificity, but additional investigations, including further comparisons with established NE markers, should confirm these primary results.

The aim of the present study was to explore the diagnostic value of INSM1 as an NE marker in pulmonary tumors by using IHC staining but also analysis of INSM1 mRNA levels.

**MATERIALS AND METHODS**

**Study Populations**

The present study included 654 surgically resected primary lung cancers from 646 individuals (8 cases with 2 synchronous primary lung cancers each) originally included in 3 independent nonselective cohorts from the Uppsala Lung Cancer Study (resected 2006–2010), the Southern Swedish Lung Cancer Study (2005–2011), and the Malmö Diet and Cancer Study (1992–2010). Missing cases as compared to previous publications were due to insufficient tumor tissue remaining in the tissue microarray (TMA) cores.

All 3 cohorts nonselectively included surgically treated primary lung cancer cases except that CTs were only included in the Malmö Diet and Cancer cohort. All cases were updated in accordance with the 4th edition of the WHO classification on lung cancer in 2015, and the results of several non-NE IHC markers including thyroid transcription factor 1 (TTF-1), napsin A, p40, and cytokeratin 5 and the results of several non-NE IHC markers including thyroid transcription factor 1 (TTF-1), napsin A, p40, and cytokeratin 5 were previously briefly presented, but all cases were reevaluated in the present study. As part of the present investigation, 2 cases were reclassified: one as LCNEC (previous NE stainings from the INSM1 clone EPR12044(B) (Abcam, Cambridge, United Kingdom) as well, but owing to insufficient staining quality in the test runs, this antibody was omitted. For most of the extra SCLC cases, synaptophysin clone SP11 (Ventana Medical Systems, Tucson, Arizona) was used instead of MRQ-40 owing to change of clone available in the clinical setting. The staining of control tissue was identical for the 2 clones.

The fraction of positive viable tumor cells was scored by using 5 categories: 0% (no positive tumor cells), less than 10%, 10% to 24%, 25% to 49%, and 50% or more. Weak to strong staining intensity was considered positive. All markers were evaluated by a pathologist (H.B.).

For adenosquamous carcinoma and combined LCNEC, the 2 cell populations were evaluated separately. The AC component of these cases was morphologically and immunohistochemically similar to “pure” pulmonary AC and therefore grouped together with the AC cases. The same applied to the SqCC component of the adenosquamous carcinomas and “pure” pulmonary SqCC. NSCLC cases with expression of NE markers were reviewed again to ascertain the histologic type.

Receiver operating characteristics (ROC) analysis was used to identify the best cutoff for the different IHC NE markers to separate NE tumors from NSCLC. Student t test and Mann-Whitney U test were used for comparison of groups and \( \chi^2 \) for investigation of relationships. The analyses were performed with MedCalc Statistical Software version 14.12.0 (MedCalc Software, Ostend, Belgium).

### Table 1. Staining Specifications

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Vendor</th>
<th>Dilution</th>
<th>Pretreatment</th>
<th>Control Tissue</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD56</td>
<td>123C3</td>
<td>Agilent/Dako (Glostrup, Denmark)</td>
<td>1:50</td>
<td>CC1 + Amp</td>
<td>Appendix, tonsil, liver</td>
<td>Predominantly membranous</td>
</tr>
<tr>
<td>Chromogranin A</td>
<td>LK2H10</td>
<td>Cell Marque (Rocklin, California)</td>
<td>1:50</td>
<td>CC2</td>
<td>Pancreas, small intestine, tonsil</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td>INSM1</td>
<td>A-B</td>
<td>Santa Cruz Biotechnology (Dallas, Texas)</td>
<td>1:100</td>
<td>CC1</td>
<td>Pancreas, small intestine</td>
<td>Nuclear</td>
</tr>
<tr>
<td>Synaptophysin</td>
<td>MRQ-40</td>
<td>Ventana Medical Systems (Tucson, Arizona)</td>
<td>RTU</td>
<td>CC1 + Amp</td>
<td>Pancreas, small intestine, tonsil</td>
<td>Cytoplasmic</td>
</tr>
</tbody>
</table>

Abbreviations: Amp, amplification; CC1, Ventana Cell Conditioning 1 (EDTA, pH 8); CC2, Ventana Cell Conditioning 2 (citrate, pH 6); INSM1, insulinoma-associated protein 1; RTU, ready-to-use.

* Synaptophysin clone SP11 was used for most of the extra small cell lung carcinoma cases (not in tissue microarrays).
Systematic Review of the Literature

A systematic search for articles on IHC of NE markers in lung cancer was performed. The search was limited to peer-reviewed full articles in English published in the last 15 years (2004–March 2019). Studies where the results of IHC staining were used for diagnosis (eg, biopsies or cytology with no confirmation on resected material) were excluded unless only cases with obvious morphology were included, not to exclude studies on, for example, typical SCLC. To exclude small case series, where the demonstrated frequencies may potentially be less representative, we only collected data from studies that investigated at least 20 cases of a specific histologic type. PubMed was searched by using “(small cell carcinoma OR large cell neuroendocrine carcinoma OR carcinoid OR non-small cell carcinoma OR adeno-neuroendocrine OR squamous cell carcinoma) AND lung AND (synaptophysin OR CD56 OR chromogranin OR INSM1)” as search term. Further searches from reference lists were also performed.

Gene Expression Analyses

Comparisons of IHC data with gene expression of respective gene were performed in 2 cohorts. The first cohort comprised 199 surgically resected lung specimens analyzed by RNA sequencing reported by Djureinovic and coworkers, with histologic subtypes updated according to WHO 2015 guidelines. Gene expression data (fragments per kilobase million [FPKM]) were obtained from Gene Expression Omnibus as series GSE81089 and used for comparisons versus IHC scores. In this cohort, 192 cases overlapped with the patient samples analyzed by IHC (Uppsala Lung Cancer Study). The second cohort was reported by Karlsson and coworkers, comprising 159 tumors of all histologic subtypes. In this cohort, 40 of 66 RNA-sequenced LCNEC cases were also investigated in 66 RNA-sequenced LCNEC cases reported by George and coworkers. Analyses of differential gene expression of the investigated genes across clinical or histologic groups were performed by using Kruskal-Wallis test.

Ethics

The analyses of the cohorts were conducted in adherence to the Declaration of Helsinki and were approved by the regional ethical review boards in Uppsala (Dnr 2012/532) and Lund (Dnr 2004/762 and 2008/702, and Dnr 2007/445, 2008/35 and 2014/748, respectively).

RESULTS

Expression of NE Markers in Lung Cancer

The lung cancer cases available for evaluation included 54 NE tumors consisting of 24 SCLCs, 23 LCNECs (including 6 combined LCNECs with an AC component), and 7 CTs, whereof 5 typical CTs and 2 atypical CTs. The 632 NSCLC cases consisted of 421 ACs (including AC component of 5 of the combined LCNECs and 8 adenosquamous carcinomas), 197 SqCCs (including SqCC component of 6 of the adenosquamous carcinomas), 8 large cell carcinomas, and 6 sarcomatoid carcinomas. Note that here and in the following analyses and tables, each component of a mixed tumor was evaluated as a separate case, and that the AC component of 1 mixed LCNEC and the SqCC component of 2 adenosquamous carcinomas were not evaluable on the TMA. Details for expression of the NE markers CD56, chromogranin A, INSM1, and synaptophysin for the different lung cancer types are found in Table 2. Examples of NE staining in NE tumors is found in Figure 1, A through O.

ROC analyses identified any number of positive tumor cells (ie, >0) as the optimal cutoff for CD56, chromogranin A, and INSM1 to separate the NE tumors from the NSCLC cases, while at least 10% positive tumor cells was the best cutoff for synaptophysin. The sensitivity and specificity for the markers are presented in Table 3. For further analyses, “at least 10% positive tumor cells” was selected as the cutoff for a positive staining for all markers, and is used below when not otherwise stated, since it has been commonly used in the clinical setting and is recommended in the 2015 WHO classification.

Coexpression of NE Markers

Among the NE tumors, all CTs were diffusely positive for all 4 NE markers. Five of the 23 LCNEC cases (22%) were positive for only 1 NE marker, whereof 3 for CD56 only and 2 for synaptophysin only. Of the remaining 18 LCNEC cases, 3 were positive for only 2 NE markers, and 15 for 3 or all 4 markers. Three of the 24 SCLCs were negative for all NE markers, while 1 case was positive for CD56 only and another for CD56 and INSM1 only. The remaining 19 SCLC cases were positive for 3 or all 4 markers. Thus, 17% (4 of 24) of the SCLCs were positive for 1 or no NE markers. Coexpression of NE markers in NE tumors is also presented in Figure 2, A.

Among NSCLCs, more than 1 positive NE marker in the same case was seen for 6 ACs and 1 large cell carcinoma, while there were no such cases of SqCC or sarcomatoid carcinoma. Thus, 2 or more positive NE markers were seen in 1% (7 of 632) of the NSCLC cases (see Figure 2, B).

The large cell carcinoma case mentioned above was positive for 3 markers (CD56 showed negativity). Molecular analysis (from the clinical setting or previous research studies) with targeted next-generation sequencing did not reveal any EGFR, KRAS, or RB1 mutation (but did contain a TP53 mutation).

Of the 6 ACs with more than 1 positive NE marker, 3 exhibited clear gland formations. In all these 3 cases, 3 NE markers showed positivity, while napsin A and TTF-1 also showed positivity in 2 of them. In 1 of the cases, the morphology suggested a minor area with NE differentiation but insufficient for the diagnosis of combined LCNEC based on all whole tumor slides. The other 3 AC cases with more than 1 positive NE marker exhibited solid growth only. In these 3 cases, 2 or 4 NE markers showed positivity, and they all showed positivity for TTF-1 but negativity for napsin A. None of the 3 cases had EGFR or RB1 mutations, while 1 had a KRAS mutation (c.34G>T). The latter case had presence of mucin inclusions on mucin staining, but not 5 in each of 2 high-power fields (criteria for solid AC with mucin), while another case among the 3 had abundant mucin inclusions. Images of AC with positive NE markers including these 2 cases are seen in Figure 3, A through R.

If using greater than 0 positive tumor cells instead of at least 10% as the cutoff for a positive staining, 2 or more positive NE markers were seen in 21 of 23 LCNECs (91%), 22 of 24 SCLCs (92%), and 22 of 632 NSCLCs (3%).

Homogeneity of INSM1 Staining

In 29 of the 30 INSM1-positive lung cancers (97%) included in the TMA6s (excluding SCLC cases with whole tumor sections), tumor cells positive for INSM1 were seen in all TMA cores. Also, the frequency of INSM1-positive NSCLC cases was similar in the cohorts with 2 (Uppsala Lung Cancer Study and Malmö Diet and Cancer Study) and...
3 (Southern Swedish Lung Cancer Study) tissue cores in the TMAs—4 of 430 (0.9%) and 2 of 194 (1.0%), respectively ($\chi^2 P = .99$)—and the IHC scores for INSM1 were not statistically different in NSCLC cases (mean, 0.04 versus 0.05; Student $t$ test and Mann-Whitney $U$ test both $P = .5$).

Correspondingly, INSM1 showed positivity in 11 of 15 (73%) and 6 of 11 (55%) high-grade NE tumors in the cohorts with 2 and 3 tissue cores in the TMAs, respectively ($\chi^2 P = .87$), and again with nonsignificant different INSM1 scores (mean, 2.9 versus 2.1; Student $t$ test and Mann-Whitney $U$ test, $P = .20$ and $P = .14$, respectively).

**Expression of NE Markers in Metastases**

The 54 examined metastases of corresponding lung cancer cases evaluated in the Uppsala Lung Cancer Study cohort (eg, intrapulmonary, lymph node, and brain metastases) consisted of 43 ACs and 11 SqCCs, all from different individuals. There was 1 case positive for chromogranin A, 2 for CD56, 1 for INSM1, and 8 for synaptophysin. All positive cases were ACs. In 2 metastases, more than 1 NE marker was positive, just as in their primary tumors. In 3 cases, there was a discrepancy in staining result between the metastasis and the primary tumor, all regarding synaptophysin and in 1 case also CD56. After complementary staining of whole tumor sections, the difference remained for synaptophysin in only 1 case.

The 419 metastases to the lungs evaluated for INSM1 expression included 271 colorectal carcinomas, 42 renal cell carcinomas, 27 breast cancers, 17 non-SqCC gynecologic carcinomas of various origin, 17 non-SqCC gastrointestinal carcinomas of various (noncolorectal) origin, 11 prostatic carcinomas, 11 SqCCs of various origin, 8 urothelial carcinomas, 6 adenoid cystic carcinomas of various origin, 5 thymomas, 3 thyroid carcinomas, and 1 basal cell carcinoma. A positive INSM1 staining was seen only in 2 ACs from cervix (both from the same patient) and 1 ductal breast cancer. Less than 10% positive tumor cells (often only few scattered cells) were seen in an additional 26 metastases mainly originating from colon/rectum (19 cases) and less commonly from breast, cervix, vulva, small intestine, gall bladder, or tonsil (the only non-AC case).

**Review of the Literature**

The literature search identified 39 articles on IHC staining of NE markers fulfilling the criteria (see Materials and Methods),3–6,8–15,17–19,27–50. In Table 4, the accumulated data from these and the current investigation are presented, also
Figure 1. Representative images of positive neuroendocrine markers in a case of carcinoid tumor (A, D, G, J, and M), large cell neuroendocrine carcinoma (B, E, H, K, and N), and small cell lung carcinoma (C, F, I, and O). A through C, Hematoxylin-eosin. D through F, CD56. G through I, Chromogranin A. J through L, Insulinoma-associated protein 1 (INSM1). M through O, Synaptophysin. Note the appearance of INSM1 in cells with crush artefacts (L) and the varying intensity between cases (data for intensity not systematically collected) (original magnification ×40 objective [A through O]).
specifying data for any/at least 1% (any positivity and ≥1% stained tumor cells were here considered equal) and at least 10% stained tumor cells as the cutoff for a positive staining, respectively (data not provided in all studies). More details on the identified articles are found in Supplemental Table 1 (see the supplemental digital content at www.archivesofpathology.org in the September 2020 table of contents).

### INSM1 RNA Expression

To investigate the correlation between INSM1 protein and corresponding gene expression, samples from the Uppsala Lung Cancer Study (n = 192 cases) and the Southern Swedish Lung Cancer Study (n = 40) with matched mRNA data were analyzed. Spearman correlation between INSM1 gene expression and IHC scores was 0.315 in the Uppsala Lung Cancer Study and 0.50 in the Southern Swedish Lung Cancer Study data (Figure 4, A and B). Stratification by tumor histology showed that INSM1 gene expression was clearly associated with an NE histology in both cohorts (Figure 4, C and D). INSM1 showed high gene expression correlation with synaptophysin (Pearson correlation = 0.78) and CD56 (Pearson correlation = 0.85), while lower with chromogranin A (Pearson correlation = 0.47) in the Uppsala Lung Cancer Study. In the Southern Swedish Lung Cancer study, corresponding values were 0.79 for CD56 and 0.93 for chromogranin A (synaptophysin was not present on the arrays).

### DISCUSSION

The accurate identification of pulmonary NE tumors presents a diagnostic challenge. While SCLCs and CTs are often easy to diagnose, and relevant differential diagnoses may typically be excluded with a few IHC stainings, LCNEC is often more demanding and may be difficult to separate from both NSCLC and SCLC. The clinical diagnostics basically relies on adequate tissue sampling, the pathologist’s experience to recognize NE morphology, and informative IHC markers.

In our study, INSM1 was numerically the best NE marker based on ROC analysis (see Table 3), while synaptophysin was the most sensitive, especially for LCNEC (see Table 2). However, CD56 showed a comparable sensitivity, stained more SCLC cases, and had a better specificity than synaptophysin. Chromogranin A had a limited sensitivity, but a high specificity similar to that of INSM1. The accumulated data in published studies support CD56 as the best marker to identify LCNEC and SCLC (see Table 4), notably in contrast to the recommendation to use synaptophysin and chromogranin A in the WHO classification.

### Table 3. Sensitivity and Specificity for Immunohistochemical Staining of Neuroendocrine Markers to Separate Pulmonary Neuroendocrine Tumors (n = 54) From Non–Small Cell Lung Carcinomas (n = 632)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Any Positivity</th>
<th>10% Positive Tumor Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td>CD56</td>
<td>91%</td>
<td>95%</td>
</tr>
<tr>
<td>Chromogranin A</td>
<td>69%</td>
<td>97%</td>
</tr>
<tr>
<td>INSM1</td>
<td>91%</td>
<td>98%</td>
</tr>
<tr>
<td>Synaptophysin</td>
<td>93%</td>
<td>83%</td>
</tr>
</tbody>
</table>

Abbreviations: AUC, area under curve (of receiver operating characteristics analysis); INSM1, insulinoma-associated protein 1.

* The AUC value for synaptophysin is for at least 10% positive tumor cells (any positivity for the other markers).
Figure 3. Example of adenocarcinomas with more than 1 positive neuroendocrine marker, whereof 2 with only solid growth (B, E, H, K, N, and Q; and C, F, I, L, O, and R, the latter with a KRAS mutation). A through C, Hematoxylin-eosin. D through F, Periodic acid–Schiff with diastase. G through I, CD56. J through L, Chromogranin A. M through O, Insulinoma-associated protein 1 (INSM1). P through R, Synaptophysin (original magnification ×40 objective [A through R]).
### Table 4. Neuroendocrine Markers in Lung Cancer, With Positive/Total Number of Cases and (in Parentheses) Number of Studies and Range in Individual Investigations From 15 Years (Studies With INSM1 Published in 2015–2019)

<table>
<thead>
<tr>
<th>Marker</th>
<th>CD56</th>
<th>Chromogranin A</th>
<th>INSM1</th>
<th>Synaptophysin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Without regard to cutoff for positive staining</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>516/552 = 93% (8; 83%–100%)</td>
<td>546/558 = 98% (8; 93%–100%)</td>
<td>224/256 = 88% (3; 79%–100%)</td>
<td>516/526 = 98% (7; 94%–100%)</td>
</tr>
<tr>
<td>LCNEC</td>
<td>379/440 = 86% (8; 61%–94%)</td>
<td>243/440 = 55% (8; 42%–85%)</td>
<td>85/147 = 58% (4; 42%–91%)</td>
<td>301/440 = 68% (8; 55%–88%)</td>
</tr>
<tr>
<td>SCLC</td>
<td>643/712 = 90% (15; 63%–100%)</td>
<td>350/633 = 55% (14; 4%–83%)</td>
<td>419/471 = 89% (8; 75%–100%)</td>
<td>497/632 = 79% (14; 52%–100%)</td>
</tr>
<tr>
<td>NSCLC (any type)</td>
<td>321/3936 = 8% (21; 0%–28%)</td>
<td>45/1654 = 3% (16; 0%–41%)</td>
<td>12/738 = 2% (5; 0%–3%)</td>
<td>231/1716 = 13% (16; 0%–72%)</td>
</tr>
<tr>
<td>AC</td>
<td>73/1505 = 5% (14; 0%–22%)</td>
<td>301/440 = 68% (7; 94%–100%)</td>
<td>22/1058 = 4% (6; 0%–13%)</td>
<td>211/2082 = 10% (10; 3%–56%)</td>
</tr>
<tr>
<td>SqCC</td>
<td>142/1495 = 9% (15; 0%–20%)</td>
<td>59/1573 = 4% (17; 0%–26%)</td>
<td>5/414 = 1% (5; 0%–4%)</td>
<td>88/1691 = 5% (17; 0%–43%)</td>
</tr>
</tbody>
</table>

| **10% positive tumor cells as cutoff for positive staining** |       |                |       |               |
| CT               | No data (all <20 cases) | No data (all <20 cases) | 56/64 = 88% (1; 88%) | No data (all <20 cases) |
| LCNEC            | 52/70 = 74% (2; 52%–85%)  | 71/88 = 81% (2; 75%–83%)  | 66/103 = 64% (3; 57%–79%) | 46/70 = 66% (2; 55%–87%) |
| SCLC             | 54/102 = 53% (3; 36%–63%) | 6/786 = 0.8% (0%–1%)  | 220/1551 = 14% (8; 1%–69%) | 66/103 = 64% (3; 57%–79%) |
| NSCLC (any type) | 75/1231 = 6% (7; 0%–66%) | 5/544 = 1% (2; 0%–1%)  | 103/741 = 14% (5; 4%–33%) | 41/461 = 9% (5; 0%–21%) |
| AC               | 7/616 = 1% (4; 0%–3%)  | 0/228 = 0% (0%; 0%)  | 0/298 = 0% (0%; 0%)  | 0/298 = 0% (0%; 0%) |
| SqCC             | 4/251 = 2% (3; 0%–2%)  | 0/228 = 0% (0%; 0%)  | 0/228 = 0% (0%; 0%)  | 0/228 = 0% (0%; 0%) |

| **1% or any positive tumor cells as cutoff for positive staining** |       |                |       |               |
| CT               | 412/437 = 94% (5; 83%–100%) | 430/437 = 98% (5; 94%–100%) | 224/256 = 88% (3; 79%–100%) | 441/448 = 98% (97%–100%) |
| LCNEC            | 180/210 = 86% (5; 61%–94%)  | 104/210 = 50% (5; 42%–57%)  | 91/147 = 62% (4; 42%–91%)  | 145/210 = 69% (5; 61%–100%) |
| SCLC             | 351/378 = 93% (8; 70%–100%) | 235/371 = 63% (7; 34%–83%)  | 396/444 = 89% (7; 81%–98%) | 305/371 = 82% (7; 52%–100%) |
| NSCLC (any type) | 184/1973 = 9% (8; 4%–28%) | 102/2162 = 5% (10; 0%–33%) | 22/1069 = 2% (5; 0%–4%)  | 211/2082 = 10% (10; 3%–56%) |
| AC               | 50/821 = 6% (5; 3%–15%)  | 35/861 = 4% (6; 0%–41%)  | 18/652 = 3% (4; 2%–3%)  | 142/785 = 18% (6; 7%–72%) |
| SqCC             | 130/1052 = 12% (6; 5%–20%) | 53/1081 = 5% (7; 0%–26%)  | 6/367 = 2% (4; 0%–4%)  | 60/1059 = 6% (7; 1%–43%) |

**Abbreviations:** AC, adenocarcinoma; CT, carcinoid tumor; INSM1, insulinoma-associated protein 1; LCNEC, large cell neuroendocrine carcinoma; NSCLC, non–small cell lung carcinoma; SCLC, small cell lung carcinoma; SqCC, squamous cell carcinoma.

**Note:** Only studies with at least 20 cases of a specific histologic type are included, and only studies reporting 10% or any/1% positive tumor cells as cutoff are included in the mid and lower parts of the table, respectively.
choice if CT is suspected, but otherwise its routine use in the clinical setting should be reconsidered given its limited sensitivity.

When the 10% cutoff for a positive staining is applied, INSM1 displayed a relatively low sensitivity for LCNEC, but more data are needed as this is based on rather few cases from just 2 studies including ours. However, the use of any positivity as a cutoff results in a more limited specificity especially for synaptophysin. It should be considered if the 10% cutoff is more applicable to CD56 and synaptophysin.

Figure 4. Gene expression analysis of insulinoma-associated protein 1 (INSM1). Cases of adenocarcinoma (AC), adenosquamous carcinoma, large cell carcinoma (LCC), large cell neuroendocrine carcinoma (LCNEC), sarcomatoid carcinoma (SARC), small cell lung carcinoma (SCLC), and squamous cell carcinoma (SqCC). (A) Spearman correlation analysis of INSM1 immunohistochemistry score versus INSM1 gene expression (FPKM) in 192 cases from the Uppsala Lung Cancer Study cohort. (B) Spearman correlation analysis of INSM1 immunohistochemistry score versus INSM1 gene expression (log2 expression) in 40 cases from the Southern Swedish Lung Cancer Study cohort. (C) INSM1 gene expression (FPKM) versus tumor histology in cases from the Uppsala Lung Cancer Study cohort. (D) INSM1 gene expression (log2 expression) versus tumor histology in cases from the Southern Swedish Lung Cancer Study cohort. Abbreviations: FPKM, fragments per kilobase million; IHC, immunohistochemistry.
while any positivity should be used for INSM1 and chromogranin A, although different cutoffs may be confusing in the clinical pathology setting.

As no marker has perfect sensitivity, it may be advisable to use a couple of markers in cases with NE morphology. The presence of 2 positive IHC NE markers also strongly suggests an NE tumor, but may also occur in NSCLC. In our literature search, 8% (149 of 1777) of NSCLCs were positive for at least 2 of CD56, chromogranin A, and synaptophysin (see Supplemental Table 1), but it is noteworthy that the frequency was less than 2% in 3 studies (including our study; see Figure 2, B) 10,27 while it was exceedingly high in 2 studies (19% and 55%), which led to the overall high occurrence.29,32 The frequency of positive NE markers is reported to be rather high in non-NE components of combined LCNEC or SCLC.54

Recently, it has been debated whether staining with NE markers should be performed in all poorly differentiated NSCLC cases (in addition to AC and SqCC markers),5,25,56 but since there is no consistent evidence of clinical relevance,29,32,43,47 it is advisable to apply IHC NE markers only when NE morphology is suggested.1,16,56 If strictly limiting to obvious NE morphology, it would probably be advantageous to use the most sensitive IHC NE markers (tentatively CD56 in combination with synaptophysin and/or INSM1). However, it is currently not clear how often pathologists suggest NE morphology in NSCLC cases, but it is probable that markers with high specificity will lead to fewer misclassified cases in the clinical setting. Also, how often pathologists fail to notice NE morphology when present is equally important, and further studies are needed for strong evidence when IHC NE markers should be performed.

Napsin A was previously considered not to be expressed in LCNEC, but recent reporting of napsin A positivity in LCNEC,25,57 and cases that are borderline AC–LCNEC,58 or borderline basoloid SqCC–LCNEC,59 questions its diagnostic use. In our borderline cases (solid AC with more than 1 positive NE marker) we included data from previous mutation analyses, as it may be argued that RB1 mutations (or loss) favor a true NE lineage (“SCLC-like”), while cases with KRAS/STK11/KEAP1 or PI3K/AKT/mTOR mutations more resemble NSCLC on a molecular level (“NSCLC-like”).29,60,63 Similarly, genetic alterations commonly differ between CTs and high-grade NE tumors,64 which theoretically could be of aid in borderline CT–LCNEC cases.64

In our analysis of 232 cases with matched INSM1 gene expression and protein data we observed a generally good correlation between RNA and immunopositivity, and high INSM1 gene expression in high-grade NE tumors. The level of RNA–protein correlation and elevated gene expression in NE tumors for INSM1 was on a par with similar data/levels for CD56, chromogranin A, and synaptophysin. These findings suggest that INSM1 is an equally good marker of NE tumors also on the gene expression level and may be used in, for example, gene expression–based predictors of NE tumor histology. Indeed, in a gene expression network analysis of lung cancer, INSM1 was identified to be a component of a neuroendocrine metagene together with, for example, synaptophysin and chromogranin A.65 Moreover, we demonstrate that gene expression of INSM1 is also associated with a SCLC-like molecular phenotype of LCNEC reported by George and coworkers,66 indicating that INSM1 positivity may be a potentially useful future marker also in this context.

The main limitation of our study is that TMAs are not perfectly comparable to whole tumor sections and provide limited information on tumor heterogeneity (although our comparisons of cores suggest this might not be a significant problem for INSM1). However, the size resembles that of biopsy specimens quite well. From the diagnostic point of view, the most relevant study material for pulmonary tumors would probably be biopsy and cytologic specimens from cases with diagnosis verified on resected material, but TMAs enable evaluation of much larger materials. There were a few cases with missing data in our material due to tissue running out in the TMA blocks, which is unlikely to affect the general results. Since we evaluated NE markers, it might have been advantageous with enrichment of NE tumors, including metastases of pulmonary NE tumors to other organs, in the cohorts instead of nonselective resected cancers. However, nonselective cohorts result in more valid specificity and predictive values, and as such our study complements previously published investigations with enrichment of NE tumors.3,4,9

In summary, there is strong support to include INSM1 as a recommended NE marker in pulmonary pathology. CD56 in combination with INSM1 and/or synaptophysin appear to be the best panel of NE markers, especially for high-grade NE tumors. Further studies are needed on INSM1 in LCNEC and on the cutoff (any positivity versus 10%) for a positive staining.

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