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Original contribution

Insulinoma-associated protein 1 immunostaining on cytology specimens: an institutional experience☆,☆☆

Erika F. Rodriguez MD, PhD[a,*], J. Judd Fite MD, MBA[a], Sayanan Chowsilpa MD[a,b], Zahra Maleki MD[a]

[a]*Department of Pathology, Division of Cytopathology, The Johns Hopkins Hospital, Baltimore, MD 21287, USA*
[b]*Department of Pathology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand*

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Summary Neuroendocrine tumors (NETs) are epithelial neoplasms with prominent neuroendocrine differentiation. Cytologic examination and utilization of immunohistochemical (IHC) markers are important diagnostic tools for the evaluation of these tumors. Herein we report our experience with the application of INSM1 in cytology samples. We searched our pathology system for cytologic specimens with INSM1 IHC performed from 2017 to 2018. Patients' demographics were recorded, and cytology materials were reviewed including all neuroendocrine IHC markers performed. A total of 134 (67 male, 67 female) specimens with INSM1 IHC were identified. Specimens included 91 (68.2%) NETs or tumors with neuroendocrine features (TNEFs), 33 (24.3%) nonneuroendocrine lesions (non-NET), and 10 (7.5%) nonneoplastic diagnoses. INSM1 was positive in 90 (99%) of the NET/TNEFs and negative in 32 (97%) non-NETs. CD56 was positive in 42 (95.5%) of the NET/TNEFs and negative on 9 (69.2%) of the non-NETs. The sensitivity of INSM1 was 99% and specificity was 97%, whereas the sensitivity of CD56 was 95.5% and specificity was 69.2%. Chromogranin had the lowest sensitivity (82.5%), and synaptophysin had the lowest specificity (66.7%). Both positive and negative predictive values of INSM1 were higher than CD56 (99% versus 91.3% and 97% versus 81.8%, respectively). INSM1 is a sensitive and specific marker for detection of NETs in cytology samples independent of primary site.

1. Introduction

Neuroendocrine tumors (NETs) are defined as epithelial neoplasms with prominent neuroendocrine differentiation [1]. NETs are relatively uncommon neoplasms with an incidence of 5.86/100 000 per year and a prevalence estimated at 103.312 cases in the United States [2]. However, according to recent studies, there has been an increase in incidence of NET in the United States and other countries [3-5]. NET incidence has been increasing at all sites, stages, and grades [3].

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* Corresponding author at: Department of Pathology, Johns Hopkins University, Carnegie 469—Pathology, 600 North Wolfe St, Baltimore, MD 21287.
E-mail address: erodri17@jhmi.edu (E. F. Rodriguez).

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Although NET can be seen in a wide age range, the highest increase in the incidence is seen in patients 65 years or older [3]. Although NETs are relatively rare neoplasms, the prevalence of this tumor is higher than that of gastric and pancreatic adenocarcinomas combined [6]. Diagnosis of NET remains a diagnostic problem in daily pathology practice.

When focusing on clinical outcome, NETs demonstrate variable biological behavior. NET can arise from any organ. However, it is more common in the gastrointestinal tract and the lung [7]. Several nomenclature systems have been used to report this group of neoplasms [1,8] with the intention of stratifying prognostic groups of NET. As a general rule, NETs are divided into well-differentiated tumors (low grade or intermediate grade) and poorly differentiated tumors (high-grade neuroendocrine carcinomas) such as small cell carcinoma.

Cytologic evaluation is an accepted method to diagnose NETs [9,10]. Cytology specimens are considered superior to tissue biopsies in the diagnosis of small cell carcinoma of the lung (SCLC) [11]. On cytology specimens, well-differentiated tumors are characterized by the presence of a monomorphic population of small- to medium-sized cells distributed singly and in loosely cohesive aggregates. The neoplastic cells have smooth nuclear membranes, stippled chromatin, and eccentrically placed nuclei [9]. In contrast, poorly differentiated neuroendocrine carcinomas are characterized by neoplastic cells with prominent atypia, as well as the presence of necrosis, mitosis, and apoptotic bodies.

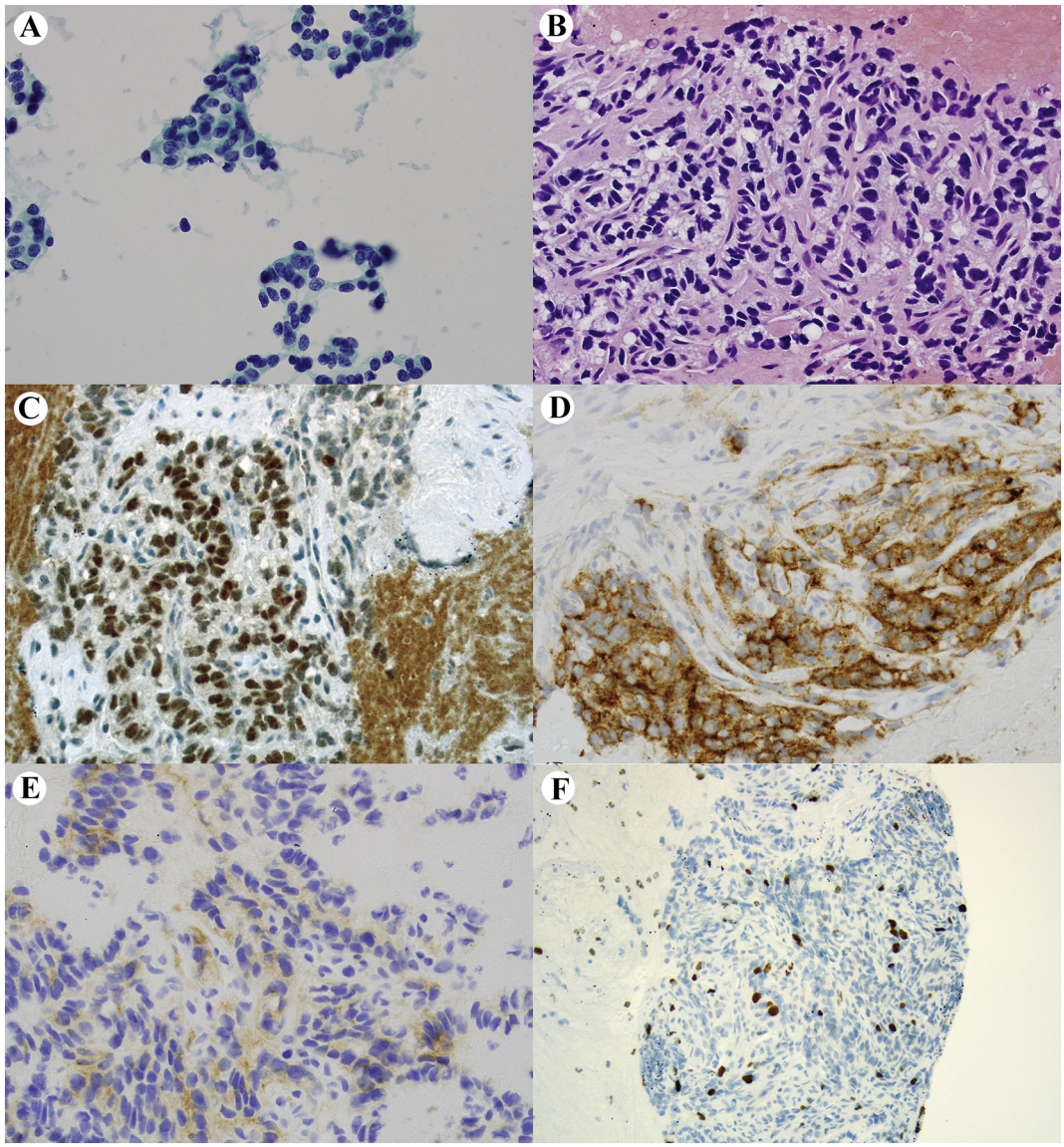

Fig. 1 Well-differentiated pancreatic NET. A, Clusters of loosely cohesive of relatively uniform cells are seen. The chromatin is stippled, and the cytoplasm is scant to moderate (Papanicolaou stain, original magnification ×400). B, Cell block shows a tissue fragment of neuroendocrine cells with no necrosis or mitotic figures (H&E, ×200). C, INSM1 highlights NET cells, nuclear staining (IHC, ×400). D, CD56 is positive in NET cells, cytoplasmic staining (IHC, ×400). E, Synaptophysin is positive in NET cells, cytoplasmic staining (IHC, ×400). F, Well-differentiated pancreatic NET. Ki-67 shows a very low proliferation index (IHC, ×400).

The cells are usually small- to medium-sized and exhibit nuclear molding. The classic stippled chromatin is also present [9-11]. Although there is discordance in the literature regarding the need to use immunohistochemical (IHC) neuroendocrine markers, the application of stains has been recommended. In fact, it improves diagnostic accuracy when it is used along with morphologic features [1,12-14].

Insulinoma-associated protein 1 (INSM1) has been reported as a valuable stain for neuroendocrine differentiation [15-18]. Herein, we compare the sensitivity and specificity of INSM1 in NET with other neuroendocrine markers and the performance of the antibody according to site and differentiation of the tumor.

2. Materials and methods

2.1. Specimen collection

After institutional review board approval, a search of the electronic pathology database system at the Johns Hopkins Hospital was done to identify specimens where INSM1 was requested from January 2017 to July 2018. Patients' demographics were recorded. The neoplastic specimens were categorized as NET/tumors with neuroendocrine features (TNEFs) and non-NETs depending on the diagnosis. The non-neoplastic lesions were analyzed as well. NETs and TNEFs

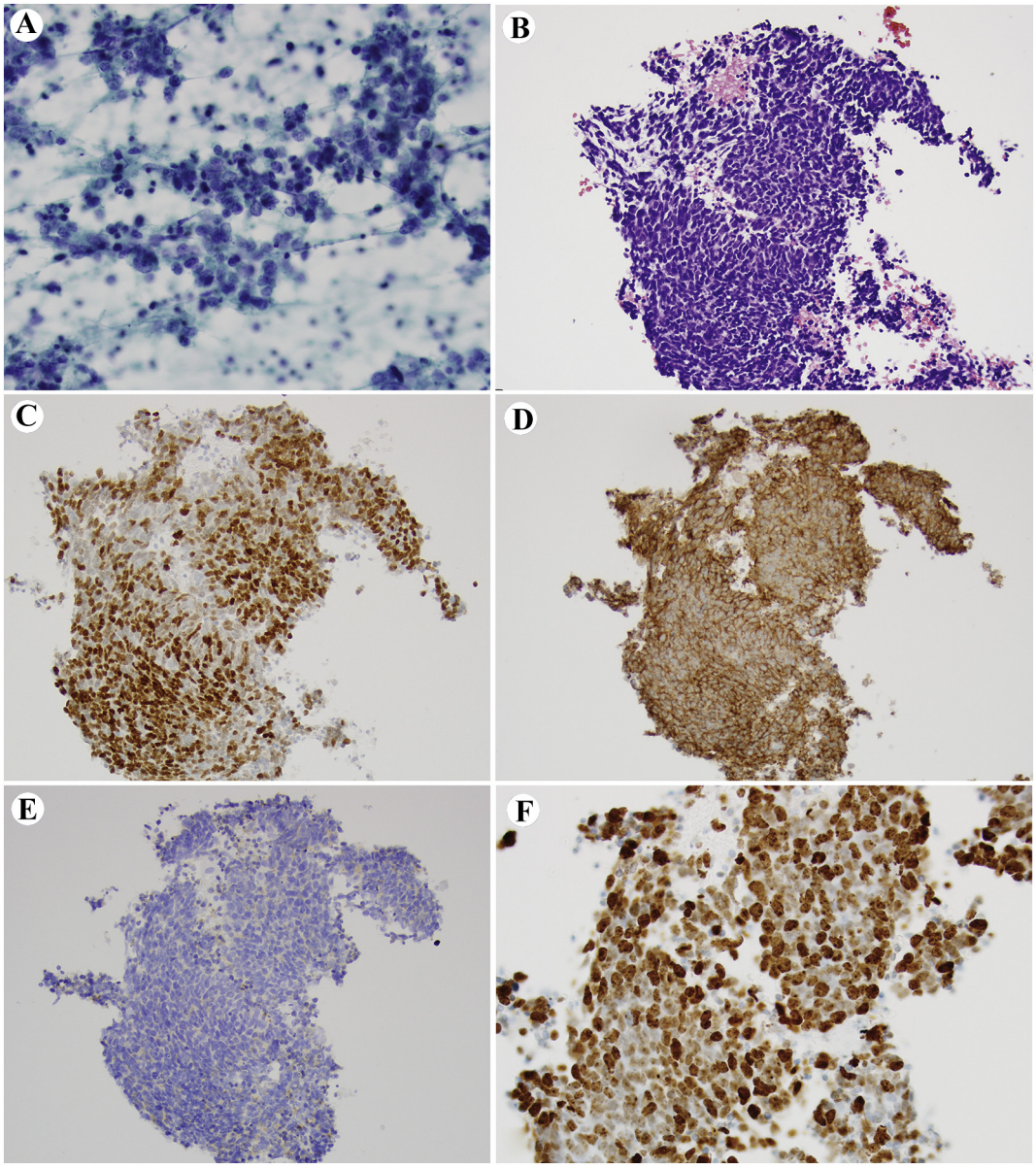

Fig. 2 Lung small cell carcinoma. A, Clusters of loosely cohesive of relatively uniform small cells are seen. The chromatin is powdery, and the cytoplasm is scant. There is focal nuclear molding and necrosis in the background (Papanicolaou stain, original magnification ×400). B, Cell block shows a tissue fragment of high-grade neuroendocrine cells (H&E stain, ×200). C, INSM1 highlights NET cells, nuclear staining (IHC, ×200). D, CD56 is positive in NET cells, cytoplasmic staining (IHC, ×200). E, Lung small cell carcinoma. Synaptophysin is negative in NET cells, cytoplasmic staining (IHC, ×200). F, Lung small cell carcinoma. Ki-67 shows a high proliferation index (IHC, ×400).

were further categorized as well-differentiated and poorly differentiated tumors following the *pathology reporting of NET* consensus [1,8]. A subset of these specimens involving the lung has been published separately [18]. All the slides, including aspirated smears stained by Diff-Quik stain, Papanicolaou stain, hematoxylin and eosin (H&E)–stained cell blocks, and IHC-stained slides, were reviewed by 2 pathologists (S. C. and Z. M.). The biopsy site, the cytology diagnosis, and IHC neuroendocrine marker studies were recorded.

2.2. IHC stains

IHC stains were performed at the discretion of the cytopathologist responsible for the case at the time of diagnosis. Staining methods were performed following the manufacturer's protocol. In summary, IHC was done on formalin-fixed, paraffin-embedded cell blocks and small core biopsies. The cell blocks were cut into 5-μm sections on positively charged glass slides. Antibodies used included the following: INSM1 (clone A8; Ventana Medical Systems, Tucson, AZ; dilution 1:200), CD56 (clone 123C3.D5; Cell Marque, Rocklin, CA/Sigma-Aldrich, St Louis, MO; prediluted), synaptophysin (clone 27G12; Novacastra/Leica Biosystems, Buffalo Grove, IL; 1:400 dilution), and chromogranin (clone LK2H10; Ventana Medical Systems; clone LK2H10; prediluted). INSM1, synaptophysin, and chromogranin stains were performed automated on BenchMark (Ventana Medical Systems).

2.3. Statistical analysis

The following statistical parameters were evaluated: sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). For the calculations, we only used the neoplastic cases.

3. Results

3.1. Demographics, collection method, and site

One hundred thirty four specimens from 134 patients (67 male, 67 female) were identified in which INSM1 IHC was performed. The patients' age ranged from 12 to 94 years (mean, 63.8 years). The specimens were collected by endobronchial ultrasound-guided fine needle aspiration (FNA; n = 48 [36%]), endoscopic ultrasound-guided FNA (n = 43

Table 1 Cases included in this study along with their INSM1 expression

Diagnosis	Cases (n = 134 [100%]), n (%)	Expression of INSM1, n (%)
NETs/TNEFs	91 (68.2)	90/91 (99)
Pancreatic NET	36 (26.7)	36/36 (100)
Small cell carcinoma	32 (24.4)	32/32 (100)
Metastatic NET	12 (9)	12/12 (100)
Poorly differentiated carcinoma with neuroendocrine features	4 (3)	3/4 (75)
High-grade neuroendocrine carcinoma	3 (2.2)	3/3 (100)
Carcinoid (pulmonary)	2 (1.5)	2/2 (100)
Merkel cell carcinoma	1 (0.7)	1/1 (100)
Metastatic urothelial carcinoma with neuroendocrine features	1 (0.7)	1/1 (100)
Non-NETs	33 (24.3)	1/33 (3)
Poorly differentiated carcinoma	10 (7.5)	0/10 (0)
Adenocarcinoma	8 (6)	0/8 (0)
Pancreatic solid pseudopapillary tumor	2 (1.5)	0/2 (0)
Acinar cell carcinoma	2 (1.5)	0/2 (0)
Squamous cell carcinoma	2 (1.5)	0/2 (0)
Non–small cell lung cancer	1 (0.7)	0/1 (0)
Urothelial carcinoma	1 (0.7)	0/1 (0)
Undifferentiated epithelial neoplasm	1 (0.7)	0/1 (0)
Clear cell sarcoma	1 (0.7)	0/1 (0)
Malignant peripheral nerve sheath tumor	1 (0.7)	0/1 (0)
Glomus tumor	1 (0.7)	0/1 (0)
Melanoma	1 (0.7)	0/1 (0)
Large B-cell lymphoma	1 (0.7)	0/1 (0)
Adrenocortical carcinoma	1 (0.7)	1/1 (100)
Nonneoplastic diagnoses	10 (7.5)	2/10 (20)
Reactive mesothelial cells	4 (3)	0/4 (0)
Pancreatic acinar cells	3 (2.2)	0/3 (0)
Pancreatic neuroendocrine cells	1 (0.7)	1/1 (100)
Parathyroid cells	1 (0.7)	0/1 (0)
Benign adrenal cells	1 (0.7)	1/1 (100)

[32%]), ultrasound-guided FNA (n = 35 [26%]), thoracentesis (n = 4 [3%]), computed tomography-guided FNA (n = 2 [1.5%]), and transthoracic FNA (n = 2 [1.5%]). The target organs were the pancreas (n = 46 [34.3%]); lymph node (n = 40 [29.9%]); liver (n = 26 [19.4%]); lung (n = 9 [6.7%]); soft tissue, abdomen, vertebra, and buttock (n = 4 [3%]); pleural fluid (n = 4 [3%]); gastric and perigastric mass (n = 2 [1.5%]); soft tissue of vagina and pelvic wall (n = 2 [1.5%]); and parotid gland (n = 1 [0.7%]).

3.2. Pancreatic NETs were the most common NET

Ninety-one cases (68.2%) were diagnosed as NET/TNEF. Most cases were pancreatic NETs (n = 36 [26.7%]), followed by SCLC (n = 32 [24.4%]) and metastatic well-differentiated NETs (n = 12 [9%]) to the liver and lymph nodes Figs. 1 and 2. Four cases of poorly differentiated carcinomas with neuroendocrine features were from the lungs [2], and one of each of the liver and lymph node. Three cases of high-grade neuroendocrine carcinomas were obtained from lymph nodes and soft tissue. Two cases of carcinoid tumors were obtained from the lung. One case was metastatic Merkel cell carcinoma to the liver, and 1 case was metastatic urothelial carcinoma with neuroendocrine features to the soft tissue. The diagnostic categories are summarized in Table 1.

3.3. Most nonneuroendocrine lesions were poorly differentiated carcinomas

Thirty-three neoplastic cases had the differential diagnosis of NET based on clinical suspicion or morphologic features. The most common tumor was poorly differentiated carcinoma (n = 10 [7.5%]) followed by adenocarcinoma (n = 8 [6%]; Table 1).

3.4. Pancreatic neuroendocrine cells (islet cells) and benign adrenal cells were immunoreactive for INSM1

On 10 cases diagnosed as nonneoplastic lesions, positivity for INSM1 was seen on pancreatic neuroendocrine islet cells (n = 1 [0.7%]) and benign adrenal cells (n = 1 [0.7%]; Fig. 3). The following cells were negative for INSM1: mesothelial cells (n = 4 [3%]), pancreatic acinar cells (n = 3 [2.2%]), and parathyroid cells (n = 1 [0.7%]).

3.5. Nearly all NET/TNEFs were immunoreactive for INSM1

INSM1 was performed on 91 cases of NET/TNEF. Only 1 case was negative for INSM1 (1%; Table 1). This particular cytology specimen was diagnosed as high-grade carcinoma

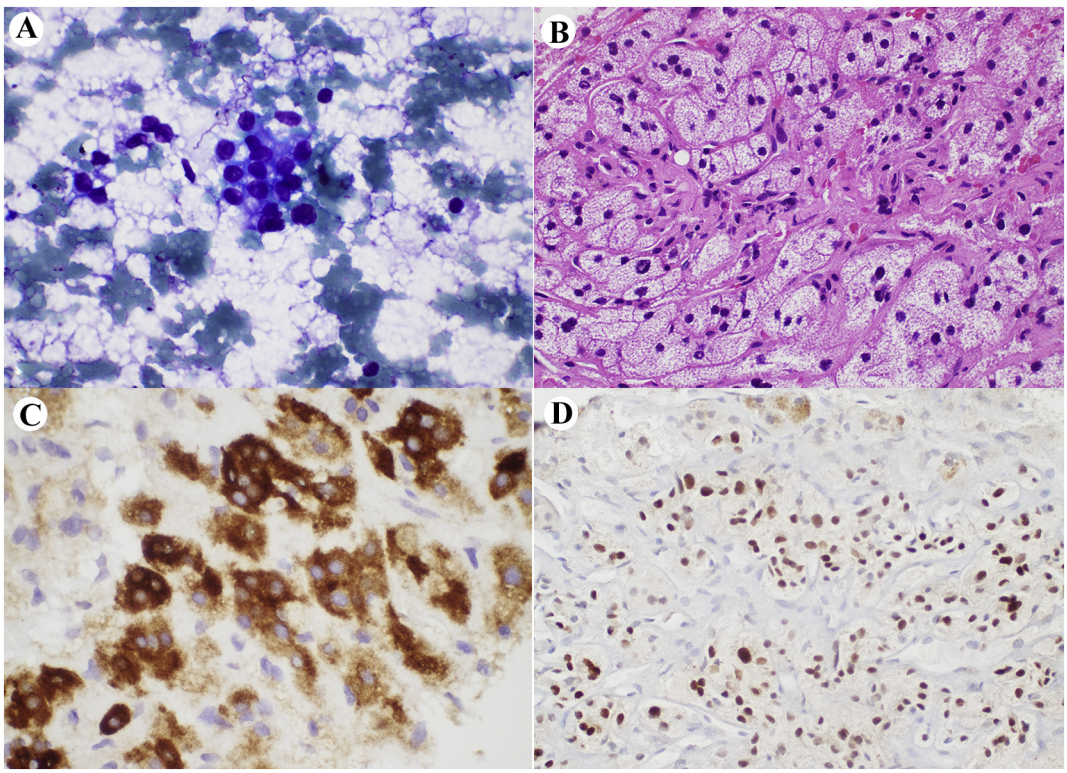

Fig. 3 Adrenal cortical cells. A, Cluster of epithelial cells with vacuolated cytoplasm (DQ stain, original magnification ×400). B, Cell block shows adrenal tissue (H&E, ×400). C, Inhibin is positive in adrenal tissue (IHC, ×400). D, INSM1 is also positive in adrenal tissue nuclei (IHC, ×400).

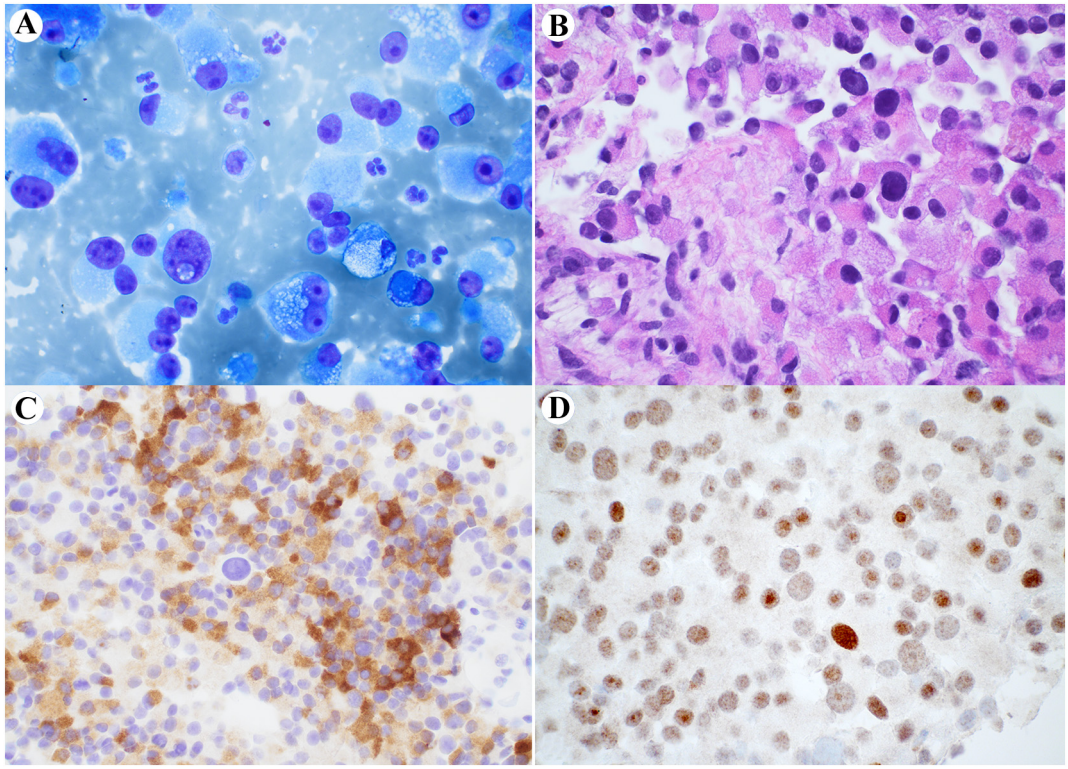

Fig. 4 Metastatic adrenocortical carcinoma. A, Cluster of neoplastic epithelial cells with vacuolated cytoplasm and prominent nucleoli (DQ stain, original magnification ×400). B, Cell block shows epithelial cells with vacuolated cytoplasm and prominent nucleoli (H&E, ×400). C, Inhibin is positive in neoplastic cells (IHC, ×400). D, INSM1 is weak/focally positive in the neoplastic cells (IHC, ×400).

with neuroendocrine features, which was focally positive for CD56 and negative for synaptophysin and chromogranin. The tumor was diagnosed as SCLC on subsequent tumor resection, which showed focal positivity for INSM1, CD56, and synaptophysin.

3.6. Nearly all non-NETs were negative for INSM1

INSM1 was performed on 33 non-NETs; it was only positive in 1 case of metastatic adrenocortical carcinoma to the liver (Table 1, Fig. 4). In this case, INSM1 expression was weak/focal, whereas synaptophysin was strong and diffuse. No other neuroendocrine markers were used. The patient did not have subsequent resection or other surgical pathology specimens at our institution. Both pancreatic solid pseudopapillary tumors were positive for synaptophysin, chromogranin, and CD56, and negative for INSM1. Also, in 1 metastatic adenocarcinoma, CD56 was positive, whereas INSM1 was negative. See Table 2 for additional details on neuroendocrine markers results.

Table 2 Staining pattern of neuroendocrine markers on cytology specimens in both NETs and non-NETs

Interpretation	Neuroendocrine markers			
	INSM1, n (%)	CD56, n (%)	Synaptophysin, n (%)	Chromogranin, n (%)
NETs	91 (100%)	44 (100)	43 (100)	39 (100)
Positive	90 (99)	42 (95.5)	41 (95.3)	32 (82.1)
Diffuse/strong	76 (84.4)	40 (95.2)	35 (85.4)	22 (68.8)
Focal/weak	14 (15.6)	2 (4.8)	6 (14.6)	10 (31.2)
Negative	1 (1)	2 (4.5)	2 (4.7)	7 (17.9)
Non-NETs	33 (100)	13 (100)	21 (100)	16 (100)
Positive	1 (3)	4 (30.8)	7 (33.3)	4 (25)
Diffuse/strong	0	4 (100)	4 (57.1)	3 (75)
Focal/weak	1 (100)	0	3 (42.9)	1 (25)
Negative	32 (97)	9 (69.2)	14 (66.7)	12 (75)

Table 3 Diagnostic value of INSM1 comparing to other neuroendocrine markers in cytology materials

Diagnostic tests	Neuroendocrine markers			
	INSM1 (%)	CD56 (%)	Synaptophysin (%)	Chromogranin (%)
Sensitivity	99	95.5	95.4	82.5
Specificity	97	69.2	66.7	75
PPV	99	91.3	85.4	88.9
NPV	97	81.8	87.5	63.2

3.7. Statistical analysis of the neuroendocrine markers

INSM1 had a sensitivity of 99% and a specificity of 97%, whereas CD56 had a slightly lower sensitivity (95.5%), with a considerably lower specificity (69.2%). Chromogranin had the lowest sensitivity (82.5%), whereas synaptophysin had the lowest specificity (66.7%). INSM1 had a PPV of 99% and an NPV of 97%. CD56 had a PPV of 91.3% and an NPV of 81.8%. Synaptophysin had a PPV of 85.4% and an NPV of 87.5%. Chromogranin had a PPV of 88.9% and an NPV of 63.2% (Table 3).

4. Discussion

INSM1 is an important transcription factor involved in the development of neural and neuroendocrine tissues [19]. INSM1 has been reported to be expressed in several NETs and neuroepithelial tumors such as SCLC, medullary thyroid carcinoma, pituitary adenoma, and medulloblastoma [17]. A recent study by Rooper et al [15] on surgical pathology samples provided data supporting that INSM1 could be used as the only neuroendocrine marker on pulmonary NET. In other studies, INSM1 performed similarly to traditional neuroendocrine markers [17]. Doxtader et al [16] and our group described the utility of INSM1 in the diagnosis of SCLC on cytology material. In the study by Doxtader and Mukhopadhyay [16], 92% of pulmonary neuroendocrine neoplasms were positive for INSM1, with 93% of SCLC being positive for INSM1. Similarly, in our study, INSM1 was positive in 97% of SCLCs [18]. In our experience with cytology material, INSM1 interpretation was easier and faster owing to its nuclear staining pattern, whereas CD56 and other neuroendocrine markers stain with a cytoplasmic pattern.

In our current study, INSM1 showed high sensitivity and specificity for the diagnosis of NET/TNEF. Moreover, INSM1 showed higher sensitivity and specificity than did CD56. The PPV and NPV of INSM1 were also higher. Conversely, chromogranin was the least sensitive neuroendocrine marker and synaptophysin was the least specific one. In addition, INSM1 was positive in all well-differentiated NET on cytology materials, similar to what Rooper et al have described on well-differentiated NET of the thoracic cavity as well as all metastatic NETs on histology specimens.

Only 1 case (1%) of poorly differentiated NET/TNEF was negative for INSM1 and positive for CD56 on cytology sampling, whereas the neoplasm was focally positive for INSM1 in the resection, as well as other neuroendocrine markers. On this particular case, the negativity for INSM1 can be explained by sample limitations. Therefore, INSM1 can be used as the only neuroendocrine marker in cytology material when the cytomorphologic features are suggestive of a NET. However, if INSM1 is negative and the main differential diagnosis based on morphology is NET, the panel may be expanded to include other neuroendocrine markers such as CD56.

In the group of non-NETs, INSM1 was positive in 1 case, which was metastatic adrenal cortical carcinoma. Adrenal cortical carcinoma is a rare tumor that has been described as negative for INSM1 [17].

Our study had few limitations, including the unequal number of stains performed in a given case. The study focused on cytology samples and the IHC studies were ordered at the sign-out by the attending pathologist. Unfortunately, the materials were often the only samples available for analysis. In addition, it is current practice in cytology to preserve neoplastic tissue for future studies that could assist with treatment options and cost savings.

In summary, INSM1 is a reliable immunostain in the detection of neuroendocrine neoplasms, and its expression is independent of site of origin and/or tumor grade. It has better sensitivity and specificity compared with those of CD56, synaptophysin, and chromogranin. A key advantage of INSM1 IHC in diagnostic practice is its clean nuclear reactivity, which is easy to interpret, a feature that is particularly suited to small samples, as they are often encountered in cytology practice.

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