





# Insulinoma-Associated Protein 1 (INSM1) Expression in Neuroendocrine Neoplasms: A Newly Discovered Diagnostic Marker

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## Abstract

**Introduction** Neuroendocrine neoplasms (NENs) are heterogeneous group of neoplasms with relatively low incidence. Diagnosis of NENs requires an integrated approach of histology, immunohistochemistry, and molecular study. In the present study, we evaluated insulinoma-associated protein 1 (INSM1) expression in NENs and correlated it with other established neuroendocrine markers.

**Materials and Method** Retrospective cross-sectional study was conducted in a tertiary care center. Consecutively, 100 cases from year November 2019 to January 2021 were enrolled in the study and all relevant data were noted.

**Results** The mean ( $\pm$ standard deviation) age of the patients was 55.5 ( $\pm$ 10.6) years with a male preponderance. Total 59% of the tumors were located in the lung of which 67% were poorly differentiated neuroendocrine carcinoma. INSM1 were positive in 97% cases, while synaptophysin (SYN) in 96% and chromogranin A (CgA) in 86%. Correlation of INSM1 expression with SYN and CgA was statistically significant ( $p$ -value  $<$  0.05). Mean H-score of INSM1 was significantly higher than SYN and CgA and it was statistically significant ( $p$ -value  $<$  0.001).

**Conclusion** In the present study, the expression of INSM1 was seen in 97% cases of NENs. A statistically significant association was found between INSM1 and traditional NE markers. As a nuclear marker it is easy to interpret and it showed higher H-score. We conclude that INSM1 is a highly sensitive marker and recommend to incorporate it in the routine practice to aid in the diagnostic workup. However, a larger cohort is required to establish the organ-specific sensitivity and specificity of INSM1.

## Keywords

- ▶ neuroendocrine neoplasms
- ▶ insulinoma-associated protein 1
- ▶ immunohistochemistry
- ▶ traditional neuroendocrine markers
- ▶ H-score

## Introduction

Neuroendocrine neoplasms (NENs) are heterogeneous group of disorders with varied histological patterns and nomenclature.<sup>1</sup> Recently, the incidence of NENs has been increased from an estimated 1.09 per 100,000 people in 1973 to 6.98 per 100,000 people in 2012 in the United States.<sup>2</sup> According to the Surveillance, Epidemiology, and End Results<sup>3</sup> and

Indian<sup>4</sup> data more than 60% of neuroendocrine tumors (NETs) arise from the gastroenteropancreatic NETs (GEP-NETs). Clinical course of NENs is different and depends upon the location of the tumor; however, a significant number of patients can present with advanced stage.<sup>5</sup> All NENs share a common neuroendocrine origin and have varied organ-specific characteristics, biological behavior, prognosis, and treatment.<sup>5</sup> Diagnosis of NENs requires an

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integrated approach of pathological, immunohistochemical, genetic, and molecular markers.<sup>5</sup>

Insulinoma-associated protein 1 (INSM1) is a zinc-finger transcription factor which has a key role in the development of neuroendocrine differentiation in various tissues.<sup>6</sup> Insulinoma-associated-1 gene encodes the INSM1, which was first discovered in 1992 at the National Institutes of Health (Bethesda, Maryland, United States) in human pancreatic insulinoma tissue and murine insulinoma cell lines.<sup>7</sup> Rosenbaum et al<sup>8</sup> reported, INSM1 as a robust immunohistochemical marker of neuroendocrine differentiation in normal and neoplastic human tissue. INSM1 is the first and the most widely validated pan-neuroendocrine marker which shows nuclear positivity.

There is a paucity of Indian literature on this new and emerging marker. In the present study, we will investigate the expression of INSM1 in NENs and compare it with the already established neuroendocrine markers.

## Materials and Methods

**Sample size and study design:** This was a retrospective cross-sectional study done at the department of oncopathology in a tertiary cancer center. Consecutive 100 cases of confirmed NENs from November 2019 to January 2021 were enrolled in the study.

**Inclusion and exclusion criteria:** Immunohistochemically proven cases of NENs were included in the study. Tumors showing neuroendocrine differentiation without immunohistochemical confirmation and inadequate tissue, suspicious lesions, and cytologically diagnosed cases were excluded.

Demographic details were retrieved from the hospital database. All tissues were fixed in 10% buffered formalin and processed for hematoxylin and eosin and immunohistochemical study. NENs are classified according to the World Health Organization classification.<sup>9</sup> Immunostaining using synaptophysin (SYN) (SP11, monoclonal antibody, Thermo-scientific, 1:50), chromogranin A (CgA) (LK2H10, monoclonal antibody, Thermo-scientific, 1:100), and INSM1 (clone: MRQ-70, rabbit monoclonal antibody, Cell Marque, 1:50) antibodies were done on all cases. Nuclear immunoreactivity for INSM1 and cytoplasmic stain for SYN and CgA in tumor cells were considered positive. For all markers, both the percentage of cells and intensity of immunoreactivity were noted. H-score assessment was done for INSM1, SYN, and CgA.<sup>10-12</sup>

## Statistical Analysis

Age, sex, location of tumor, histologic type, and histological grade were noted. Associations between categorical variables (location of tumor, tumor subtype, tumor grade) were analyzed using chi-square test. Wilcoxon rank test was used for comparison of H-score value. Two-sided *p*-values of <0.05 were considered significant. All statistical analyses were carried out using SPSS 20.

**Ethics:** The institutional review committee of the Gujarat Cancer and Research Institute approved the study, approval

number IRC/35/2019 and date November 14, 2019. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

**Table 1** Clinicopathological and immunohistochemical characteristics

Parameters	Number of cases (n = 100)
Age	
< 50 y	33
≥ 50 y	67
Site	
Lung	59
GIT + pancreas	31
Others	10
Sex	
Male	81
Female	19
Types	
NET	29
NEC	67
Combined	4
Subtypes	
NET	
NET 1	16
NET2	5
NET3	8
MINEN	1
NEC	
SMCC	65
LCNEC	2
Combined	3
Immunohistochemistry	
Synaptophysin	
Positive	96
Negative	04
Chromogranin	
Positive	86
Negative	14
INSM1	
Positive	97
Negative	03

Abbreviations: GIT, gastrointestinal tract; INSM1, insulinoma-associated protein 1; LCNEC, large cell neuroendocrine carcinoma; MINEN, mixed neuroendocrine nonneuroendocrine neoplasms; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumor; SMCC, small cell carcinoma.

## Results

Clinicohistopathological and immunohistochemical characteristics are described in ►Table 1. The mean ( $\pm$ standard deviation [SD]) age was 55.5 ( $\pm$ 10.61) years ranging from 25 to 76 years with most of the cases between 51 and 60 years (37%). Male preponderance was noted with male-to-female ratio of 4.2:1. Lung was the most frequently affected organ (59%). Total 67% cases had poorly differentiated neuroendocrine carcinoma (PD-NEC) of which 65% had small cell morphology. Total 43% of cases had a maximum tumor size of  $>$  7 cm. Total 97 cases were positive for INSM1. Of three negative cases, two were PD-NEC of the lung and one was jejenum well-differentiated NET. Of these three negative INSM1 cases, one case was positive for both SYN and CgA, while two were positive of SYN and CgA, respectively.

### Correlation of Expression of INSM1 with Tumor Characteristics

Association between various variables is described in ►Table 2. INSM1 expression was compared with size, site, histological type, histological grade, histological subtypes, and immunohistochemical markers. Statistically significant association was found between the expression of INSM1 and SYN and CgA ( $p$ -value  $<$  0.05). However, no statistically significant association was found between histological type, histological grade, and histological subtypes ( $p$ -value  $>$  0.05). Comparison of INSM1 with traditional NE markers is shown in ►Supp. Figs. S1 and S2.

## Comparisons of H-Scores

Detailed site-specific H-score calculation for all three antibodies is discussed in ►Table 3. Minimum and maximum H-score for all three antibodies was 0 and 300, respectively. Mean ( $\pm$ SD) H-score of SYN, CgA, and INSM1 was 119.65 ( $\pm$ 78.706), 108.70 ( $\pm$ 99.307), and 194.55 (77.818), respectively. Comparison of H-score is described in ►Table 4.

## Discussion

NET is a relatively rare disorder and its diagnosis requires an integrated approach. Well-established neuroendocrine markers such as SYN, CgA, and CD56 are cytoplasmic markers and it is difficult to interpret them in small biopsy. In our study, we have evaluated the newly evolved marker INSM1 and compared it with various parameters which are statistically not significant. Our findings are concordant with McHugh et al.<sup>13</sup> In their study they have compared INSM1 in GEP-NENs. Total 97% cases were positive for INSM1, which was higher than the traditional NE markers. Correlation of expression of INSM1 with SYN and CgA showed statistically significant association in our study ( $p$ -value  $<$  0.05). Rooper et al<sup>14</sup> studied INSM1 in all thoracic NETs and they have found statistically significant association of INSM1 with SYN and CgA ( $p$ -value  $<$  0.001), which was concordant with our study. However, Zou et al<sup>15</sup> did not find statistically significant association of INSM1 with CgA and SYN ( $p$ -value  $<$  0.09 and 0.494, respectively). In our study, sensitivity of INSM1, SYN, and CgA were 97, 96, and 86%, respectively. Comparison

**Table 2** Correlation of INSM1 with clinicopathological variable

Variables	Total cases	Number of cases (%)		p-Value <sup>a</sup>
		INSM1 positive	INSM1 negative	
Histological types				
NET	29	28 (96.6)	1 (3.4)	0.0591
NEC	67	65 (97.0)	2 (3.0)	
Combined	4	4 (100)	0 (0)	
Site				
Lung	59	57 (96.6)	2 (3.4)	0.841
GIT and pancreas	31	30 (96.8)	1 (3.2)	
Others	10	10 (100)	0 (0)	
SYN				
Positive	96	94 (97.9)	2 (2.1)	0.008
Negative	4	3 (75)	1 (25)	
CgA				
Positive	86	83 (96.5)	3 (3.5)	0.011
Negative	14	14 (100)	0 (0)	

Abbreviations: CgA, chromogranin A; GIT, gastrointestinal tract; INSM1, insulinoma-associated protein 1; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumor; SYN, synaptophysin.

<sup>a</sup>Chi-square test.

**Table 3** H-score comparison of SYN, CgA, and INSM1 by location of tumor

Tumor type	Antibodies	Positive cases	H-score (mean)
GIT	SYN	21/21	135
	CHR	18/21	128
	INSM1	21/21	188
Pancreaticobiliary system	SYN	9/10	144
	CHR	10/10	167
	INSM1	10/10	206
Lung NET	SYN	3/3	58
	CHRO	3/3	85
	INSM1	3/3	170
Lung SMCC	SYN	48/51	103
	CHR	43/51	80
	INSM1	49/51	194
Lung LCNEC	SYN	1/1	80
	CHR	0/1	0
	INSM1	1/1	270
Nasal cavity	SYN	1/1	90
	CHR	0/1	0
	INSM1	1/1	90
BOT	SYN	1/1	80
	CHR	1/1	120
	INSM1	1/1	190
Presternal	SYN	1/1	185
	CHR	1/1	250
	INSM1	1/1	160
UB	SYN	1/1	200
	CHR	1/1	190
	INSM1	1/1	260
Cervix	SYN	5/5	163
	CHR	4/5	74
	INSM1	5/5	198
Vault	SYN	1/1	285
	CHR	1/1	175
	INSM1	1/1	300

Abbreviations: BOT, base of tongue; CgA, chromogranin A; GIT, gastrointestinal tract; INSM1, insulinoma-associated protein 1; LCNEC, large cell neuroendocrine carcinoma; NET, neuroendocrine tumor; SMCC, small cell carcinoma; SYN, synaptophysin; UB, urinary bladder.

of sensitivity of INSM1 and conventional marker of previous study is discussed in ► **Table 5**.

In our study, we evaluated INSM1 and other conventional NE markers by calculating H-scores. The mean H-scores of INSM1, SYN, and CgA in our study were 194.5, 119.6, and 108.7, respectively. This is slightly lower than the study by Fujino et al.<sup>16</sup> In their study, the mean H-scores were 211, 191, and 122, respectively. However, in our study comparison of INSM1 H-score with traditional NE markers was

statistically significant. This result was concordant with the study of Fujino et al.<sup>16</sup> ( $p$ -value < 0.0001).

## Conclusion

INSM1 is a superior immunohistochemical marker when compared with traditional NE markers (SYN and CgA). Statistically significant association was found between expression of INSM1 and SYN and CgA. However, larger prospective

**Table 4** H-score comparison

Parameters	Number	p-Value <sup>a</sup>
SYN vs. INSM1		
SYN < INSM1	79	0.001
SYN > INSM1	18	
SYN = INSM1	3	
CgA vs. INSM1		
CgA < INSM1	76	0.001
CgA > INSM1	21	
SYN = INSM1	3	
SYN vs. CgA		
SYN < CgA	53	0.195
SYN > CgA	46	
SYN = CgA	1	

Abbreviations: CgA, chromogranin A; INSM1, insulinoma-associated protein 1; SYN, synaptophysin.

<sup>a</sup>Wilcoxon rank test.

**Table 5** Comparison of expression of NE markers with previous study

Studies	INSM1 (%)	SYN (%)	CgA (%)
Our study	97/100 (97)	96/100 (96)	86/100 (86)
Fujino et al <sup>16</sup>	100/102 (98)	88/102 (86.2)	84/102 (82.3)
Rosenbaum et al <sup>8</sup>	27/30 (90)	29/30 (96.7)	21/30 (70)
Aldera et al <sup>17</sup>	59/69 (85.5)	63/69 (91.3)	48/69 (69.5)
McHugh et al <sup>13</sup>	89/110 (82.9)	109/110 (99.1)	96/110 (87.3)
Mukhopadhyay et al <sup>6</sup>	144/152 (95)	147/150 (98)	125/149 (84)
Kriegsmann et al <sup>18</sup>	276/372 (74.2)	319/372 (85.8)	289/372 (77.7)
Rooper et al <sup>14</sup>	99/103 (96.1)	79/103 (76.7)	67/103 (65.0)
Sakakibara et al <sup>19</sup>	120/141 (85.1)	87/141 (61.7)	74/141 (52.5)
González et al <sup>20</sup>	32/32 (100)	32/32 (100)	31/32 (97)

Abbreviations: CgA, chromogranin A; INSM1, insulinoma-associated protein 1; NE, neuroendocrine; SYN, synaptophysin.

studies should be undertaken to assess the site-based INSM1 expression as well as to investigate the role of INSM1 in prognosis of NEN.

#### Funding

None.

#### Conflict of Interest

None declared.

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