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Comparison of insulinoma-associated protein 1 (INSM1) with traditional neuroendocrine markers in gastrointestinal and pancreatic mixed neuroendocrine-non-neuroendocrine neoplasms (MiNENs)

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Abstract

The traditional diagnostic markers for mixed neuroendocrine-non-neuroendocrine neoplasms (MiNENs) are synaptophysin (SYP), chromogranin A (CHGA) and CD56. However, there is still a lack of a large series of article focused on the expression of insulinoma-associated protein 1 (INSM1) in gastrointestinal and pancreatic MiNENs. This study compared the expression of INSM1 and traditional neuroendocrine markers in MiNENs. In this study, we collected 46 cases of gastrointestinal and pancreatic MiNENs and performed immunohistochemical staining for INSM1, SYP, CHGA, and CD56. Histologically, the neuroendocrine components of MiNENs were all neuroendocrine carcinomas, with small cell neuroendocrine carcinomas accounting for 15.2% (7/46) and large cell neuroendocrine carcinomas accounting for 84.8% (39/46). With respect to immunohistochemical expression, the overall sensitivity of INSM1 was 80.4% (37/46), which was lower than that of SYP (100%, 46/46), but comparable to that of CHGA (67.4%, 31/46) or CD56 (73.9%, 34/46). The overall specificity of INSM1 was 91.3% (42/46), which was greater than that of SYP (63.0%, 29/46) and CD56 (69.6, 32/46), but was not significantly different from that of CHGA (82.6%, 38/46). The proportion of 3 + staining for SYP (100%, 46/46) was greater than that of INSM1 (71.7, 33/46), while the proportion of 3 + staining for CHGA (10.9, 5/46) or CD56 (21.7, 10/46) was lower than that of INSM1. In conclusion, INSM1 exhibited high sensitivity and specificity in the diagnosis of gastrointestinal and pancreatic MiNENs.

Keywords Mixed neuroendocrine-non-neuroendocrine neoplasm, Synaptophysin, Chromogranin A, Neuroendocrine carcinoma, Insulinoma-associated protein 1

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Introduction

Mixed neuroendocrine-non-neuroendocrine neoplasms (MiNENs) are epithelial tumours with a mixture of neuroendocrine components and non-neuroendocrine components, each accounting for at least 30%. The neuroendocrine component and non-neuroendocrine component in MiNENs should be clearly distinguished based on histology and immunohistochemistry. Commonly used neuroendocrine markers include synaptophysin (SYP), chromogranin A (CHGA) and CD56. In digestive system MiNENs, the neuroendocrine component is usually neuroendocrine carcinoma (NEC), while well-differentiated neuroendocrine tumours are very rare [1]. NEC can be divided into small cell neuroendocrine carcinoma (SCNEC) and large cell neuroendocrine carcinoma (LCNEC). The non-neuroendocrine components of MiNENs are typically adenocarcinoma, squamous cell carcinoma, acinar cell carcinoma, or any other definable tumour category, as appropriate [2].

The insulinoma-associated protein 1 (INSM1) gene encodes a DNA-binding protein with five zinc finger domains and is highly evolutionarily conserved. INSM1 expression exhibits strict tissue specificity. Early studies using in situ hybridization revealed that INSM1 transcripts were specifically expressed in the forebrain, midbrain, hindbrain, olfactory epithelium, retina, cerebellum, pancreas, thymus, thyroid, adrenal gland, and gastrointestinal tract neuroendocrine cells during foetal development [3]. INSM1 can be expressed in neuroendocrine neoplasms at multiple anatomical sites, such as the lung [4–6], digestive system [7–9], head and neck [10], skin [11, 12], prostate [13], and uterus [14]. To date, large studies on INSM1 expression in MiNENs of the gastrointestinal tract and pancreas are lacking. The main purpose of this study was to analyse the sensitivity and specificity of INSM1 expression in the neuroendocrine components of gastrointestinal and pancreatic MiNENs.

Materials and methods

Sample selection

This study was approved by the Fujian Provincial Hospital institutional review board for the protection of human subjects (Protocol code K2023-01-005). All patients were selected from the pathological database of Fujian Provincial Hospital from January 2011 to December 2022. According to the fifth edition of the World Health Organization (WHO) digestive system tumour definition of MiNENs (MiNENs are mixed epithelial neoplasms in which a neuroendocrine component is combined with a non-neuroendocrine component, each of which is morphologically (poorly formed trabeculae or sheets of poorly differentiated cells) and immunohistochemically (SYP and/or CHGA expression) recognizable as a discrete component and constitutes > 30% of the neoplasm), and excludes neuroendocrine and non-neuroendocrine coexpression tumours and collision tumours, all patients did not receive neoadjuvant chemotherapy. A total of 46 MiNEN cases were identified, including 1 case in the oesophagus, 17 cases in the oesophagogastric junction, 23 cases in the stomach, 2 cases in the duodenum, 2 cases in the pancreas, and 1 case in the colon.

Immunohistochemical analysis

All tumours in each case were collected and each FFPE block of each tumour was subjected to IHC staining. The sections were cut at 4 μm and deparaffinized. IHC was conducted via the Roche Benchmark XT automated system (Roche, Basel, Switzerland) using INSM1, CD56, SYP and CHGA antibodies (antibody clones and protocols in Supplementary material Table 1). The FFPE block containing the boundary zone between the neuroendocrine component and non-neuroendocrine component was subjected to Ki-67 IHC staining (antibody clones and protocols in Supplementary material Table 1). The percentage of tumour cells stained was scored in quartiles. No expression (Fig. 1A) was defined as 0, a value less than 10% (Fig. 1B) are defined as 1+, a value of 10–50% (Fig. 1C) are defined as 2+, and a value greater than 50% (Fig. 1D) are defined as 3+. The expression of neuroendocrine markers was defined as positive.

Statistical analysis

SPSS 27.0 software (IBM, Armonk, USA) and Prism 10.1 software (GraphPad Software, California, USA) were used for the statistical analyses. Fisher's exact test or chi-square test was used to compare the sensitivity and specificity of different neuroendocrine markers and to analyse categorical variables. $P < 0.05$ was considered to indicate statistical significance.

Results

Clinical and pathological characteristics of 46 cases of gastrointestinal and pancreatic MiNENs (Table 1)

Histological analysis of MiNENs revealed that all of the neuroendocrine components were neuroendocrine cancer (NEC). Small cell neuroendocrine carcinoma (SCNEC) accounted for 15.2% (7/46) and large cell neuroendocrine carcinoma (LCNEC) accounted for 84.8% (39/46). The non-neuroendocrine components included adenocarcinoma (42/46), squamous cell carcinoma (2/46), and acinar cell carcinoma (2/46). The degree of differentiation for adenocarcinoma or squamous cell carcinoma was mainly moderately differentiated (Supplementary material Table 4). Adenocarcinomas of the oesophagogastric junction and the stomach were tubular adenocarcinomas. We evaluated lymph node metastasis in all cases and found that 37 cases had lymph node metastasis, including 5 cases (13.5%) with pure

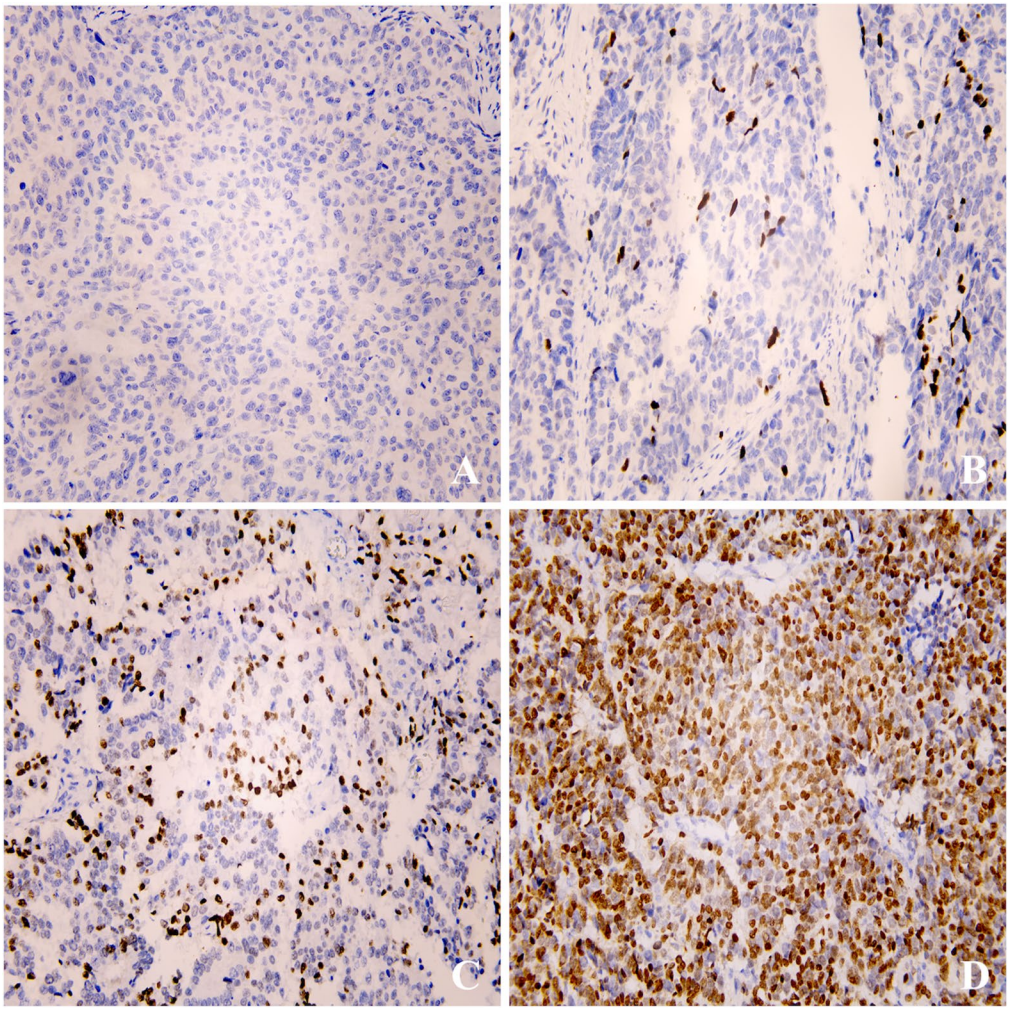

Fig. 1 Representative immunohistochemical expression levels of INSM1 in neuroendocrine components of gastrointestinal and pancreatic MiNENs. Percent of tumor cell staining was interpreted in quartiles: 0 (**A**), <10% (**B**), 10–50% (**C**), >50% (**D**)

neuroendocrine component metastasis, 25 cases (67.6%) with pure non-neuroendocrine component metastasis, and 7 cases (18.9%) with neuroendocrine component and non-neuroendocrine component metastasis.

Expression of INSM1, SYP, CHGA, and CD56 in the neuroendocrine components of gastrointestinal and pancreatic MiNENS (Table 2)

The neuroendocrine components of the MiNENs were NECs, including 7 cases (15.2%) of SCNEC (Fig. 2A) and 39 cases (84.8%) of LCNEC (Fig. 2E). The mean for Ki-67 expression index of the neuroendocrine components was 80% (ranged from 70 to 90%) (Supplementary material Table 2).We conducted a statistical analysis of the expression of four neuroendocrine markers in SCNEC and LCNEC. For SCNEC, the expression rates of INSM1 (Fig. 2B), SYP (Fig. 2C), CHGA (Fig. 2D) and CD56 were 85.7% (6/7), 100% (7/7), 57.1% (4/7), and 85.7% (6/7), respectively. For LCNEC, the expression rates of INSM1 (Fig. 2F), SYP (Fig. 2G), CHGA (Fig. 2H) and CD56 were 79.5% (31/39), 100% (39/39), 69.2% (27/39), and 71.8% (28/39), respectively. Among all MiNEN neuroendocrine components, the overall expression rate of SYP (100%) was the highest, followed by INSM1 (80.4%), and the expression rates of CD56 (73.9%) and CHGA (67.4%) were relatively low. Among the MiNEN neuroendocrine components, the proportion of 3+SYP-stained samples was 100% (46/46), but the percentages among INSM1-, CHGA-, and CD56-stained samples were 71.7% (33/46), 10.9% (5/46), and 21.7% (10/46), respectively (Table 3). The proportion of 3+staining of SYP was significantly greater than that of INSM1 ($p < 0.001$), while the proportion of 3+staining of CHGA ($p < 0.001$) or CD56 ($p < 0.001$) was significantly lower than that of INSM1 (Fig. 4E).

Table 1 Clinical and pathological characteristics of gastrointestinal and pancreatic MiNENs ($n=46$)

Variable	Category	*n* (%)
Gender		
	Male	38 (82.6)
	Female	8 (17.4)
Age		
	≤ 35	1 (2.2)
	35 ~ 60	11 (23.9)
	>60	34 (73.9)
Primary tumour site		
	Oesophagus	1 (2.2)
	Esophagogastric junction	17 (37.0)
	Stomach	23 (50)
	Duodenum	2 (4.3)
	Pancreas	2 (4.3)
	Colorectum	1 (2.2)
Histological type (Neuroendocrine components)		
	SCNEC	7 (15.2)
	LCNEC	39 (84.8)
Histological type (Non-neuroendocrine components)		
	Adenocarcinoma	42 (91.4)
	Squamous cell carcinoma	2 (4.3)
	Acinar cell carcinoma	2 (4.3)
Lymph node metastasis		
	Pure neuroendocrine components	5 (13.5)
	Pure non neuroendocrine components	25 (67.6)
	Two components	7 (18.9)

SCNEC: Small cell neuroendocrine carcinoma; LCNEC: Large cell neuroendocrine carcinoma

Table 2 Expression of INSM1, SYP, CHGA, and CD56 in the neuroendocrine components of gastrointestinal and pancreatic MiNENs

Tumor Type	Positive No./Total No. (%)			
	INSM1	SYP	CHGA	CD56
Oesophagus				
SCNEC	1/1 (100)	1/1 (100)	0/1 (0)	1/1 (100)
Esophagogastric junction				
SCNEC	2/3 (66.7)	3/3 (100)	1/3 (33.3)	2/3 (66.7)
LCNEC	11/14 (78.6)	14/14 (100)	12/14 (85.7)	10/14 (71.4)
Gastric				
SCNEC	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)
LCNEC	18/21 (85.7)	21/21 (100)	14/21 (66.7)	15/21 (71.4)
Duodenum				
LCNEC	1/2 (50)	2/2 (100)	0/2 (0)	1/2 (50)
Pancreas				
SCNEC	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)
LCNEC	0/1 (0)	1/1 (100)	1/1 (100)	1/1 (100)
Colorectum				
LCNEC	1/1 (100)	1/1 (100)	0/1 (0)	1/1 (100)

SCNEC: Small cell neuroendocrine carcinoma; LCNEC: Large cell neuroendocrine carcinoma. SYP: Synaptophysin; INSM1: Insulinoma-associated protein 1; CHGA: Chromogranin A

Expression of INSM1, SYP, CHGA, and CD56 in non-neuroendocrine components of gastrointestinal and pancreatic MiNENS (Table 4)

Among the MiNEN cases analysed, non-neuroendocrine components were adenocarcinoma (42/46), squamous cell carcinoma (2/46), and acinar cell carcinoma (2/46). The mean for Ki-67 expression index of non-neuroendocrine components was 71% (ranged from 30 to 90%) (Supplementary material Table 2).For adenocarcinomas (Fig. 3A), the overall expression rates of INSM1 (Fig. 3B and Supplementary material Fig. 6B), SYP (Fig. 3C and Supplementary material Fig. 2A), CHGA (Fig. 3D and Supplementary material Fig. 2C) and CD56 (Supplementary material Fig. 2D) were 7.1% (3/42), 35.7% (15/42), 19.0% (8/42) and 31.0% (13/42), respectively. With respect to squamous cell carcinoma (Fig. 3E), the expression rate of INSM1 (Fig. 3F) was 50% (1/2), whereas SYP (Fig. 3G), CHGA (Fig. 3H), and CD56 were not expressed. In acinar cell carcinoma (Fig. 3I), only SYP (Fig. 3K) and CD56 staining was observed, with no expression of INSM1 (Fig. 3J) and CHGA (Fig. 3L).

Sensitivity and specificity of INSM1, SYP, CHGA, and CD56 in neuroendocrine components of gastrointestinal and pancreatic MiNENS (Table 5)

Among the neuroendocrine components of MiNENs, the overall sensitivity of INSM1 was 80.4% (37/46), which was lower than that of SYP (100%, 46/46, $p=0.003$), but comparable to that of CHGA (67.4%, 31/46, $p=0.154$) or CD56 (73.9%, 34/46, $p=0.456$)(Fig. 4A). Owing to differences in tissue morphology between SCNEC and LCNEC, we assessed the expression sensitivity of the four antibodies. For SCNEC, the overall sensitivity of INSM1 (85.7%, 6/7) was not significantly different from that of SYP (100%, 7/7, $p=0.999$), CHGA (57.1%, 4/7, $p=0.559$) or CD56 (85.7%, 6/7, $p=0.999$) (Fig. 4C). For LCNEC, the overall sensitivity of INSM1 (79.5%, 31/39) was weaker than that of SYP (100%,39/39, $p=0.005$), while there was no statistically significant difference in overall sensitivity compared with CHGA (69.2, 27/39, $p=0.437$) or CD56 (71.8%, 28/39, $p=0.599$) (Fig. 4D). Among the neuroendocrine components of MiNENs, the overall specificity of INSM1 was 91.3% (42/46), which was greater than that of SYP (63.0%, 29/46, $p=0.001$) and CD56 (69.6, 32/46, $p=0.009$), but was not significantly different from that of CHGA (82.6%, 38/46, $p=0.216$) (Fig. 4B). We also analysed the overall sensitivity and specificity of INSM1, SYP, CHGA and CD56 at a 10% cut-off value. The results

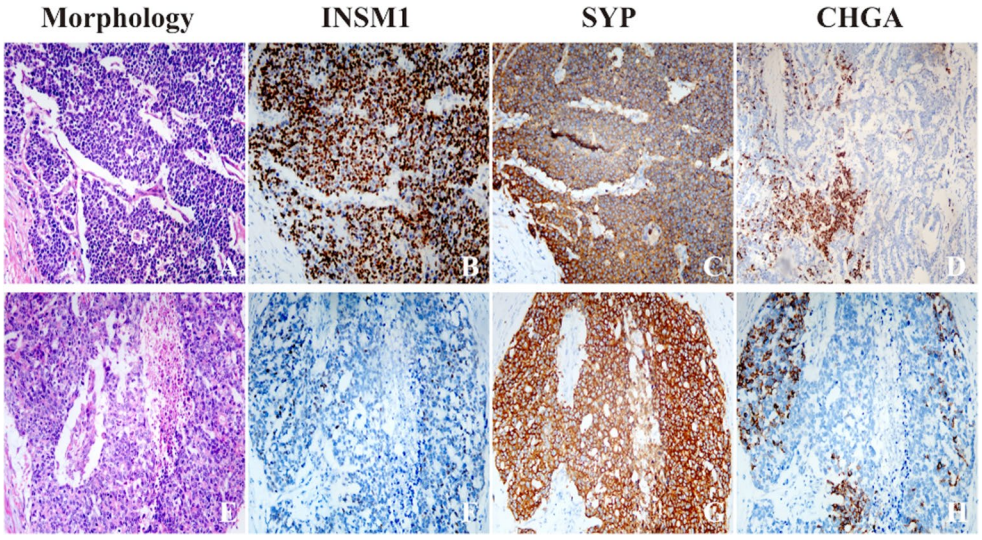

Fig. 2 Neuroendocrine component of gastrointestinal and pancreatic MiNEN is divided into SCNEC and LCNEC based on histological morphology. SCNEC displays fusiform nuclei with finely granular chromatin, and scant cytoplasm (**A**); LCNEC has round nuclei, sometimes with prominent nucleoli, and moderate amounts of cytoplasm (**E**). In our cases, SYP immunohistochemistry showed diffuse and strong positivity in both SCNEC (**C**) and LCNEC (**G**), but the expression of INSM1 (**B** and **F**) and CHGA (**D** and **H**) varied in different tumour tissues

Table 3 The staining score of INSM1, SYP, CHGA, and CD56 in neuroendocrine components of gastrointestinal and pancreatic MiNENs

Marker	No. Positive/ Total No. (%)	Immunohistochemical staining No. Positive (%)			
		0	1+	2+	3+
INSM1	37/46 (80.4)	9 (19.6)	1 (2.2)	3 (6.5)	33 (71.7)
SYP	46/46 (100)	0 (0)	0 (0)	0 (0)	46 (100)
CHGA	31/46 (67.4)	15 (32.6)	11 (23.9)	15 (32.6)	5 (10.9)
CD56	34/46 (73.9)	12 (26.1)	8 (17.4)	16 (34.8)	10 (21.7)

SYP: Synaptophysin; INSM1: Insulinoma-associated protein 1; CHGA: Chromogranin A

revealed that the overall sensitivity of INSM1 was 78.3% (36/46), less than that of SYP (100%, 46/46, $p < 0.001$), but greater than that of CHGA (43.5%, 20/46, $p < 0.001$) and CD56 (56.5, 26/46, $p=0.026$)(Supplementary material Fig. 1A and Supplementary material Table 3); the overall specificity of INSM1 was 93.5% (43/46), which was greater than that of SYP (76.1%, 35/46, $p=0.020$), but was not significantly different from that of CHGA (95.7%, 44/46, $p=0.999$) or CD56 (87.0%, 40/46, $p=0.485$)(Supplementary material Fig. 1B and Supplementary material Table 3).

Discussion

Mixed neuroendocrine-non-neuroendocrine neoplasms are tumours that differ in histological morphology and immunohistochemical expression from other neuroendocrine neoplasms and non-neuroendocrine neoplasms. The 2017 WHO Classification of Tumours of Endocrine Organs applied the term MiNEN for the first time in the classification of pancreatic tumours. Afterwards, in the fifth edition of the WHO's Digestive System Tumours, the term MiNEN was also applied to all digestive system tumour classifications. In MiNENs, the histology and immunomarker expression of neuroendocrine components are differ from those of non-neuroendocrine components. The typical immunomarkers that distinguish neuroendocrine components from non-neuroendocrine components include SYP and CHGA. SYP exhibits stronger sensitivity than the CHGA does, but the CHGA has stronger specificity [15–17]. Although the combination of two antibodies is beneficial for improving the specificity of neuroendocrine component diagnosis, SYP or CHGA is inexplicably expressed in non-neuroendocrine components. Therefore, the distinction between the neuroendocrine components and non-neuroendocrine components of MiNENs should be based not only on the expression of neuroendocrine markers but also on neuroendocrine morphology. The neuroendocrine components of MiNENs can only be identified when their immunohistochemical expression and morphology match those of neuroendocrine neoplasms. For the positivity of neuroendocrine markers in the absence of neuroendocrine morphology, the following vocabulary can be used for definition, such as carcinoma with interspersed neuroendocrine cells, amphicrine carcinoma, or carcinoma with variable or diffuse synaptophysin expression [18]. It is currently unclear whether the sensitivity and specificity of INSM1 meet the diagnostic requirements for neuroendocrine components in MiNENs. This study highlights several novel findings by analysing the clinical pathology

Table 4 Expression of INSM1, SYP, CHGA, and CD56 in non-neuroendocrine components of gastrointestinal and pancreatic MiNENs

Tumor Type	Positive No./Total No. (%)			
	INSM1	SYP	CHGA	CD56
Oesophagus				
Squamous cell carcinoma	1/1 (100)	0/1 (0)	0/1 (0)	0/1 (0)
Esophagogastric junction				
Adenocarcinoma	1/16 (6.3)	4/16 (25)	3/16 (18.8)	4/16 (25)
Squamous cell carcinoma	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
Gastric				
Adenocarcinoma	2/23 (8.7)	10/23 (43.5)	5/23 (21.7)	7/23 (30.4)
Duodenum				
Adenocarcinoma	0/2 (0)	0/2 (0)	0/2 (0)	1/2 (50)
Pancreas				
Acinar cell carcinoma	0/2 (0)	2/2 (100)	0/2 (0)	1/2 (50)
Colorectum				
Adenocarcinoma	0/1 (0)	1/1 (100)	0/1 (0)	1/1 (100)

SYP: Synaptophysin; INSM1: Insulinoma-associated protein 1; CHGA: Chromogranin A

of 46 cases of gastrointestinal and pancreatic MiNENs

Table 5 Sensitivity and specificity of INSM1, SYP, CHGA, and CD56 in neuroendocrine components of gastrointestinal and pancreatic MiNENs

	No. Positive/Total No. (%)			
	INSM1	SYP	CHGA	CD56
Overall sensitivity	37/46 (80.4)	46/46 (100)	31/46 (67.4)	34/46 (73.9)
Overall specificity	42/46 (91.3)	29/46 (63.0)	38/46 (82.6)	32/46 (69.6)
Sensitivity, SCNEC	6/7 (85.7)	7/7 (100)	4/7 (57.1)	6/7 (85.7)
Sensitivity, LCNEC	31/39 (79.5)	39/39 (100)	27/39 (69.2)	28/39 (71.8)

SCNEC: Small cell neuroendocrine carcinoma; LCNEC: Large cell neuroendocrine carcinoma. SYP: Synaptophysin; INSM1: Insulinoma-associated protein 1; CHGA: Chromogranin A

and by comparing the expression of INSM1, SYP, CHGA and CD56.

In this cohort, MiNENs mainly occurred in the stomach and oesophagogastric junction, accounting for 50% and 36.9%, respectively. In other studies, the proportion of MiNENs in the colon and rectum was the highest [19, 20], possibly as a result of differences in nationalities, environments, dietary habits or single-centre studies. Studies have shown that the overall sensitivity of INSM1 for gastrointestinal and pancreatic neuroendocrine neoplasms is 80.9–100%, with an overall specificity of 93–98% [7, 9, 15, 21–23]. The sensitivity of INSM1 in gastrointestinal and pancreatic neuroendocrine tumours

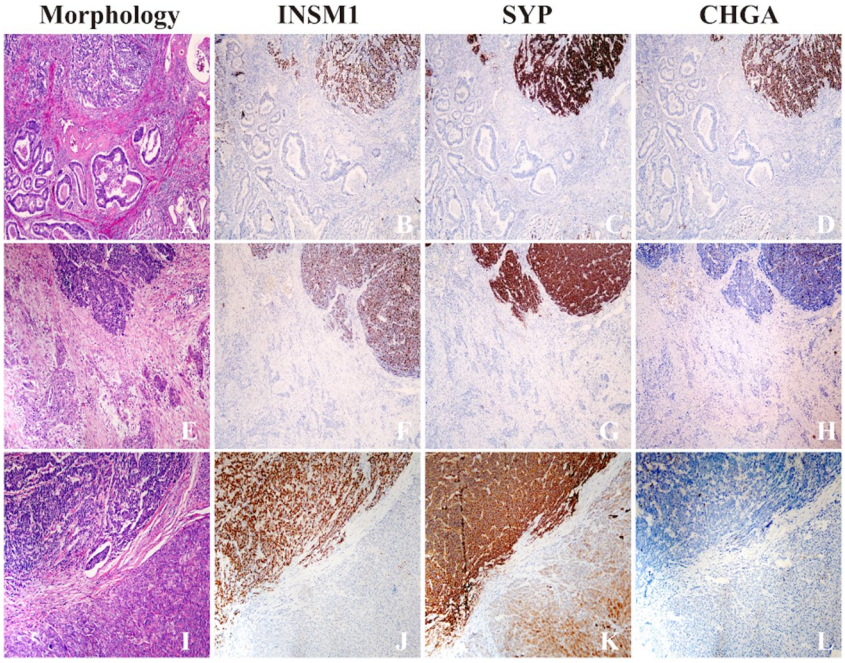

Fig. 3 In our cases, non-neuroendocrine components of MiNENs included adenocarcinoma (**A**), squamous cell carcinoma (**E**), and acinar cell carcinoma (**I**). In adenocarcinomas, the negative rates of INSM1 (**B**), SYP (**C**), and CHGA (**D**) were 92.9%, 64.3%, and 81.0%, respectively. In squamous cell carcinomas, the negative rates of INSM1 (**F**), SYP (**G**), and CHGA (**H**) are 50%, 100%, and 100%, respectively. In acinar cell carcinomas, only SYP (**K**) expression was found, while INSM1 (**J**) and CHGA (**L**) were not expressed

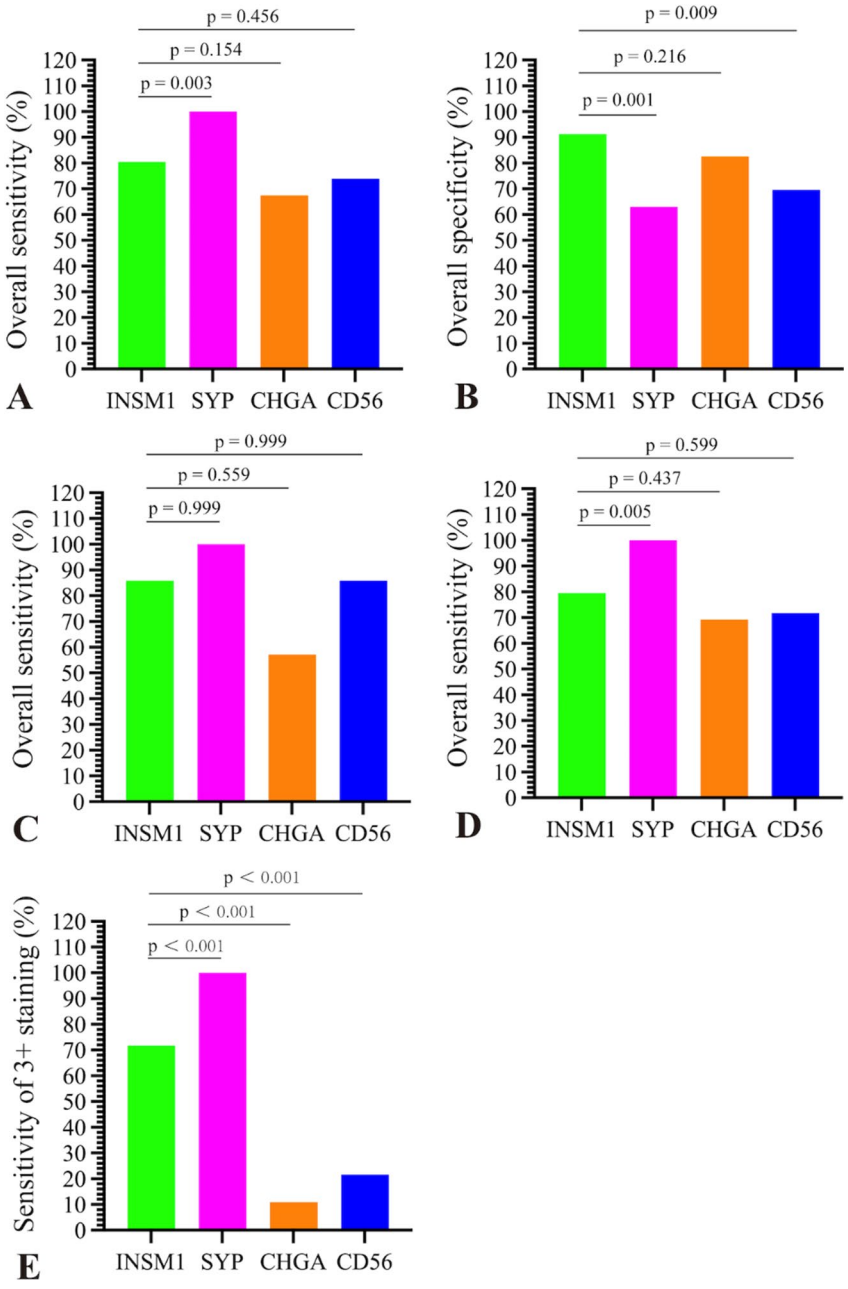

Fig. 4 Sensitivity and specificity of INSM1, SYP, CHGA, and CD56 in neuroendocrine components of gastrointestinal and pancreatic MiNENs. The overall sensitivity of INSM1, SYP, CHGA, and CD56 in neuroendocrine components of gastrointestinal and pancreatic MiNENs (**A**). The overall specificity of INSM1, SYP, CHGA, and CD56 in neuroendocrine components of gastrointestinal and pancreatic MiNENs (**B**). The overall sensitivity of INSM1, SYP, CHGA, and CD56 in SCNEC components of gastrointestinal and pancreatic MiNENs. (**C**). The overall sensitivity of INSM1, SYP, CHGA, and CD56 in LCNEC components of gastrointestinal and pancreatic MiNENs (**D**). The proportion of 3 + staining of INSM1, SYP, CHGA, and CD56 in neuroendocrine components of gastrointestinal and pancreatic MiNENs (**E**)

and neuroendocrine carcinomas is equivalent, with values ranging from 82.9 to 86.4% and 76.9–85% [15, 23], respectively. However, in these studies of INSM1, the number of MiNEN cases accounted for a small proportion and had not yet been extensively reported. By analysing the expression of neuroendocrine markers in gastrointestinal and pancreatic MiNENs, we found that the overall sensitivity of INSM1 for gastrointestinal and pancreatic MiNEN neuroendocrine components was lower than that of SYP, but comparable to that of CHGA or CD56. MiNENs are different from non-neuroendocrine neoplasms. On the one hand, MiNEN contains both neuroendocrine and non-neuroendocrine components, and both components have monoclonal origins; on the

other hand, the molecular events of MiNENs differ from those of non-neuroendocrine neoplasms [1]. In order to better elucidate the specific expression of INSM1, SYP, CHGA and CD56 in the neuroendocrine components of MiNENs, we did not select non-neuroendocrine neoplasms but rather non-neuroendocrine components of MiNENs. The results indicated that the overall specificity of INSM1 was greater than that of SYP or CD56, but was not significantly different from that of CHGA. In all MiNEN cases, the neuroendocrine components were all neuroendocrine carcinomas. Neuroendocrine carcinoma can be divided into small cell neuroendocrine carcinoma and large cell neuroendocrine carcinoma based on histological morphology. Among our cases, neuroendocrine carcinomas were mainly composed of large cell neuroendocrine carcinomas. This finding was similar to the research results of other scholars [19, 24–29]. In MiNENs, it has not been reported whether the sensitivity of INSM1 differs from that of SYP, CHGA, or CD56 due to histological differences between SCNEC and LCNEC. We found that for SCNEC, the overall sensitivity of INMS1 was comparable to that of SYP, CHGA, or CD56, while for LCNEC, the overall sensitivity of INSM1 was weaker than that of SYP and was not significantly different from CHGA or CD56.

In addition to confirming the neuroendocrine components of MiNENs, another important aspect was determining the proportion of the neuroendocrine components of MiNENs. Among our cases, SYP showed diffuse positivity in the neuroendocrine components of all MiNENs, and the proportion of 3+SYP-stained samples was greater than that of INSM1-stained samples, but the proportion of 3+staining CHGA-stained or CD56-stained samples was lower than that of INSM1-stained samples. When the non-neuroendocrine component was differentiated adenocarcinoma, due to the high sensitivity of SYP and the histological characteristics of neuroendocrine components, SYP was the most suitable indicator for evaluating the proportion of neuroendocrine components. When the non-neuroendocrine component was poorly differentiated adenocarcinoma, although SYP had higher sensitivity, INSM1 had higher specificity and a high proportion of 3+staining, and for the cases with INSM1 3+staining, especially when SYP and INSM1 expression were inconsistent, INSM1 was more suitable as an indicator to evaluate the proportion of neuroendocrine components. However, for MiNEN with poorly differentiated adenocarcinoma and INSM1 was not expressed or expressed at a low level (1+or 2+) in neuroendocrine component, the proportion of neuroendocrine component should be determined by combining the SYN positive percentage and histological characteristics of neuroendocrine neoplasms.

Our study has some limitations. First, although the analysis was used a retrospective design with a limited sample size, our sample size is the largest compared to previous reports. Second, there was relatively small number of MiNENs in the colon and pancreas. Third, Our study belonged to a single-centre research, and further validation of the sensitivity and the specificity of INSM1 in the neuroendocrine components of gastrointestinal and pancreatic MiNENs was needed from multiple centres.

In conclusion, INSM1 exhibited high sensitivity and specificity in the diagnosis of gastrointestinal and pancreatic MiNENs, and the combined application of INSM1, SYP, and CHGA was beneficial for improving the the correct diagnosis of neuroendocrine components in gastrointestinal and pancreatic MiNENs.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13000-024-01568-0.

Supplementary Material 1: Supplementary material Table 1: Immunohistochemistry methodology.

Supplementary Material 2: Supplementary material Table 2: The Ki-67 expression index of neuroendocrine components and non-neuroendocrine components of gastrointestinal and pancreatic MiNENs.

Supplementary Material 3: Supplementary material Table 3: Sensitivity and specificity of 10% cut-off for INSM1, SYP, CHGA, and CD56 in neuroendocrine components of gastrointestinal and pancreatic MiNENs.

Supplementary Material 4: Supplementary material Table 4: The degree of differentiation for adenocarcinoma and squamous cell carcinoma in gastrointestinal and pancreatic MiNENs.

Supplementary Material 5: Supplementary material Fig. 1: Sensitivity and specificity of 10% cut-off for INSM1, SYP, CHGA, and CD56 in neuroendocrine components of gastrointestinal and pancreatic MiNENs. The overall sensitivity of INSM1, SYP, CHGA, and CD56 in neuroendocrine components of gastrointestinal and pancreatic MiNENs (A). The overall specificity of INSM1, SYP, CHGA, and CD56 in neuroendocrine components of gastrointestinal and pancreatic MiNENs (B).

Supplementary Material 6: Supplementary material Fig. 2: The expression of SYP (A), INSM1 (B), CHGA (C), and CD56 (D) in adenocarcinoma.

Supplementary Material 7: Supplementary material Fig. 3: The ROC curve illustrated the diagnostic performance of INSM1 when comparing neuroendocrine components and non-neuroendocrine components in MiNENs. Area under the curve (AUC) was found for INSM1: 0.89 (95% CI, 0.82–0.97).

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None.

Author contributions

Gao rui, and Zhang Xi collected patient data and samples, and participated in the writing of the manuscript. Chen Xi, Jin Long and Lin Ying performed histopathological and immunohistochemical analyses. Yu Xunbin and Zheng Huawei performed tissue sectioning and immunohistochemical staining.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the Institutional Review Board of Fujian Provincial Hospital (protocol code No.K2023-01-005) and obtained the consent of all participants.

Competing interests
The authors declare no competing interests.

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