Am J Surg Pathol 2018;42:1503–1512

ORIGINAL ARTICLE

Mixed Adenoma Well-differentiated Neuroendocrine Tumor (MANET) of the Digestive System

An Indolent Subtype of Mixed Neuroendocrine-NonNeuroendocrine Neoplasm (MiNEN)

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BACKGROUND

2010版WHO消化系统神经内分泌肿瘤

1. 分级：根据核分裂象和KI67指数，分为3级：

	核分裂象	KI67
G1	<2个/10HPF	≤2%
G2	2--20个/10HPF	3%—20%
G3	＞20个/10HPF	≥20%

- 2. 定义：

- 神经内分泌瘤（NET）：高分化的神经内分泌肿瘤，肿瘤细胞类似于正常胃肠道内分泌细胞，有轻-中度核不典型性，核分裂象少（<20个/10HPF），分为G1和G2
- 神经内分泌癌（NEC）：低分化、高度恶性的肿瘤，核不典型性明显，多灶坏死，核分裂象多见（>20个/10HPF），分为G3
- 混合性腺神经内分泌癌(MANEC)，是指同时具有腺癌和神经内分泌癌形态特点的恶性肿瘤，每种成分至少各占30%。

- MANEC是MiNEN(Mixed neuroendocrine-nonneuroendocrine neoplasm)最常见的类型
- 而MiNEN可表现出不同的形态，非神经内分泌成分可包括腺瘤、腺癌、鳞状细胞癌、腺泡细胞癌等，神经内分泌部分也可是不同的分化程度。
- MiNEN不同的构成成分就可能产生不同的临床、病理和预后特征
- 本研究收集12例腺瘤与NET并存的混合性肿瘤，并分析其临床病理、形态、免疫表型、分子改变及预后。

MATERIALS AND MEHODS

- Cases

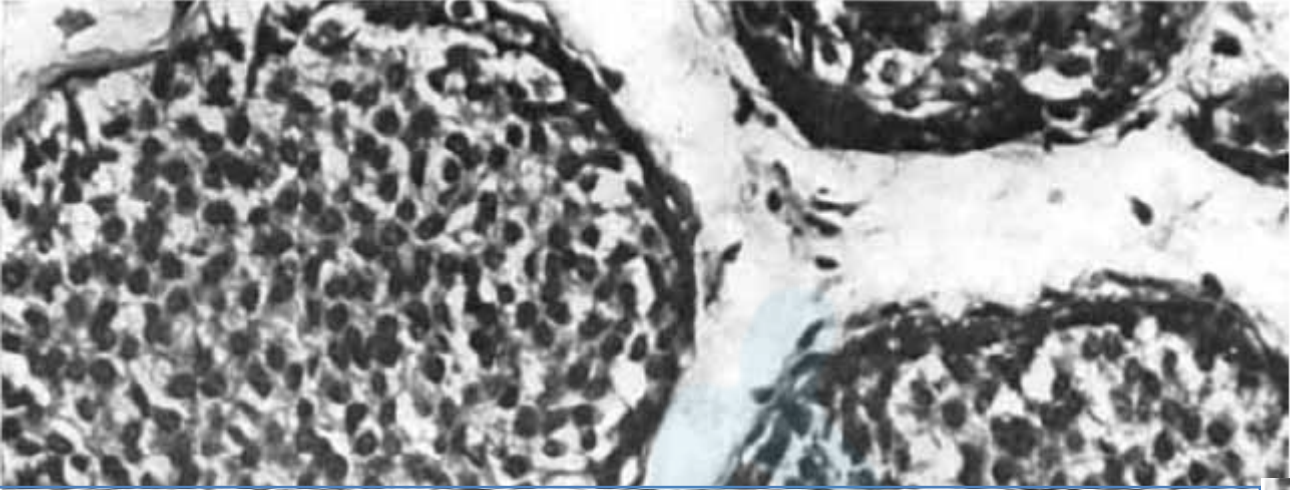
  12 mixed tumors consisting of adenoma and well-differentiated neuroendocrine tumor(MANET)of the gut were retrieved from 5 institutions ;

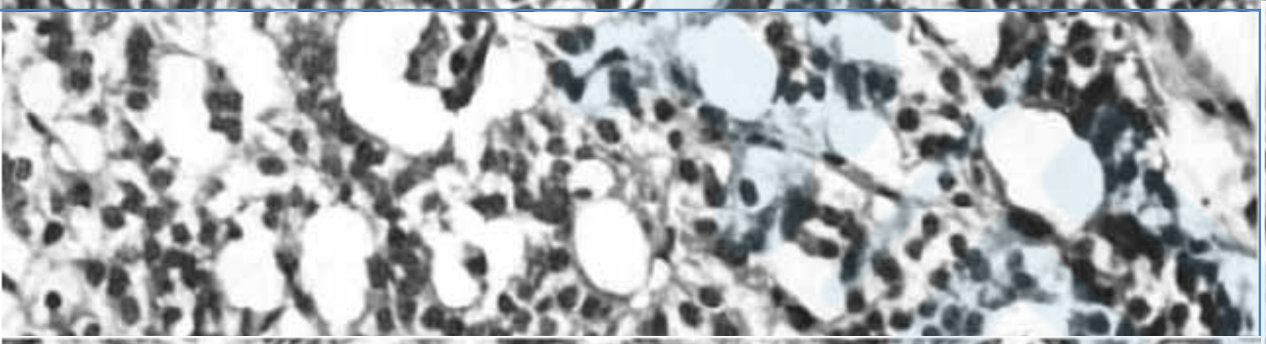
  2 cases of adenoma with neuroendocrine cell hyperplasia;
- Clinico-pathologic information: gender, age at the time of the diagnosis, symptoms, tumor site and size, presence of lymph node and/or distant metastases, follow-up data
- 1/14 surgically resected,13/14 endoscopically resected

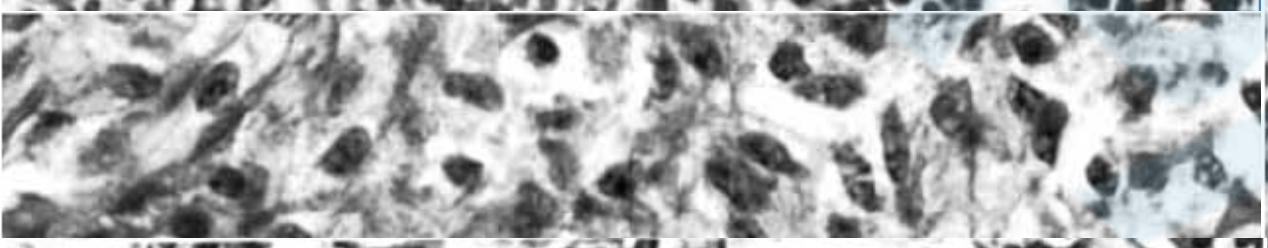
- Morphologic
  - Adenomatous
    - Type
      - Tubular
      - Tubulo-villous
      - Villous
    - Degree of dysplasia
  - neuroendocrine component
    - Architectural pattern
    - Grade
    - Level of infiltration
    - Vascular invasion

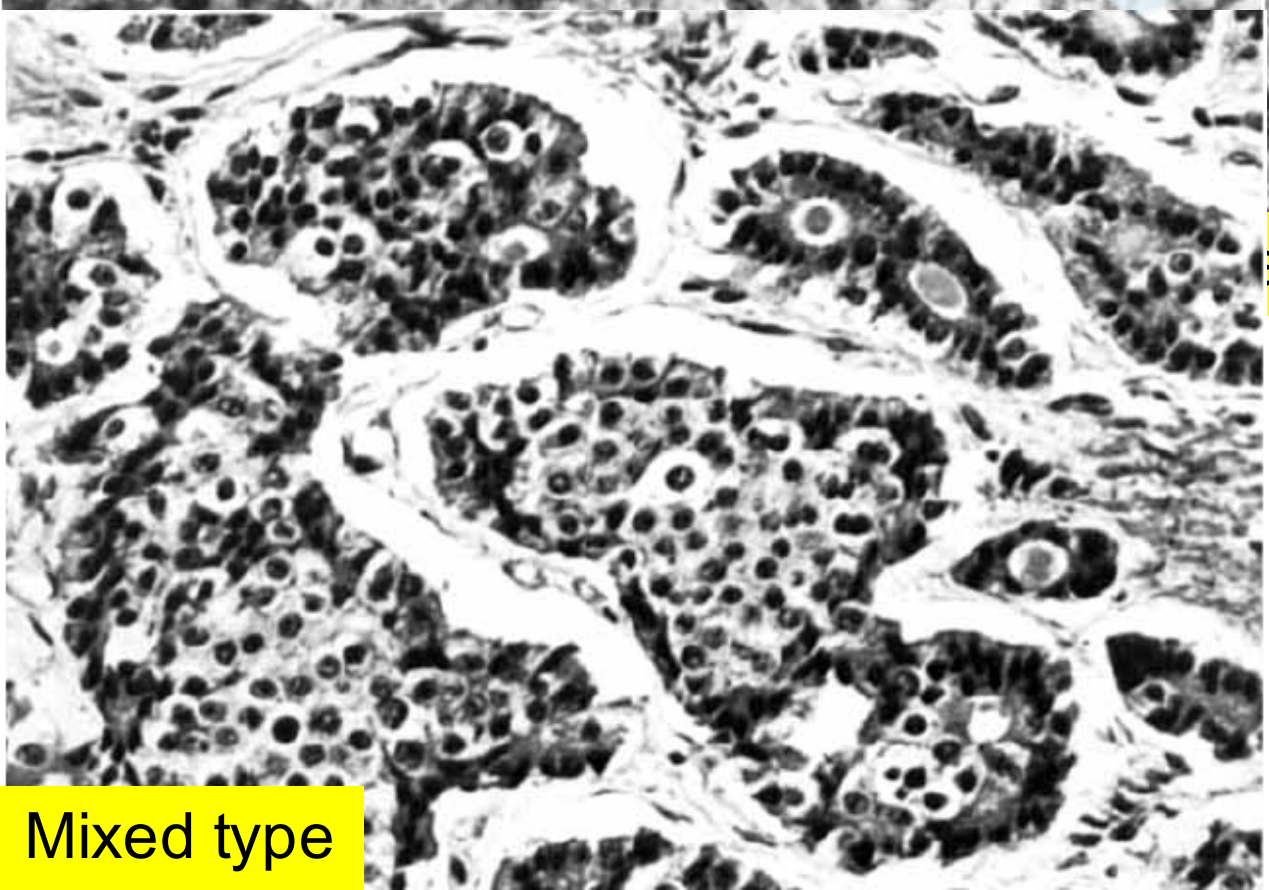
- *Soga J, Tazawa K. Pathologic analysis of carcinoids. Histologic reevaluation of 62 cases. Cancer. 1971;28:990–998.*

- **A type**: Tumors with nodular solid nests and peripheral invading cords
- **B type**: Tumors with a trabecular or ribbon-like structure forming a frequent anastomosing pattern
- **C type**: Tumors with a tubular, acinar, or rosette-like structure
- **D type**: Tumors with structurcs of lower or atypical differentiations
- **Mixed type**: Tumors with mixed strurturcs o f any combination of the above 4 types

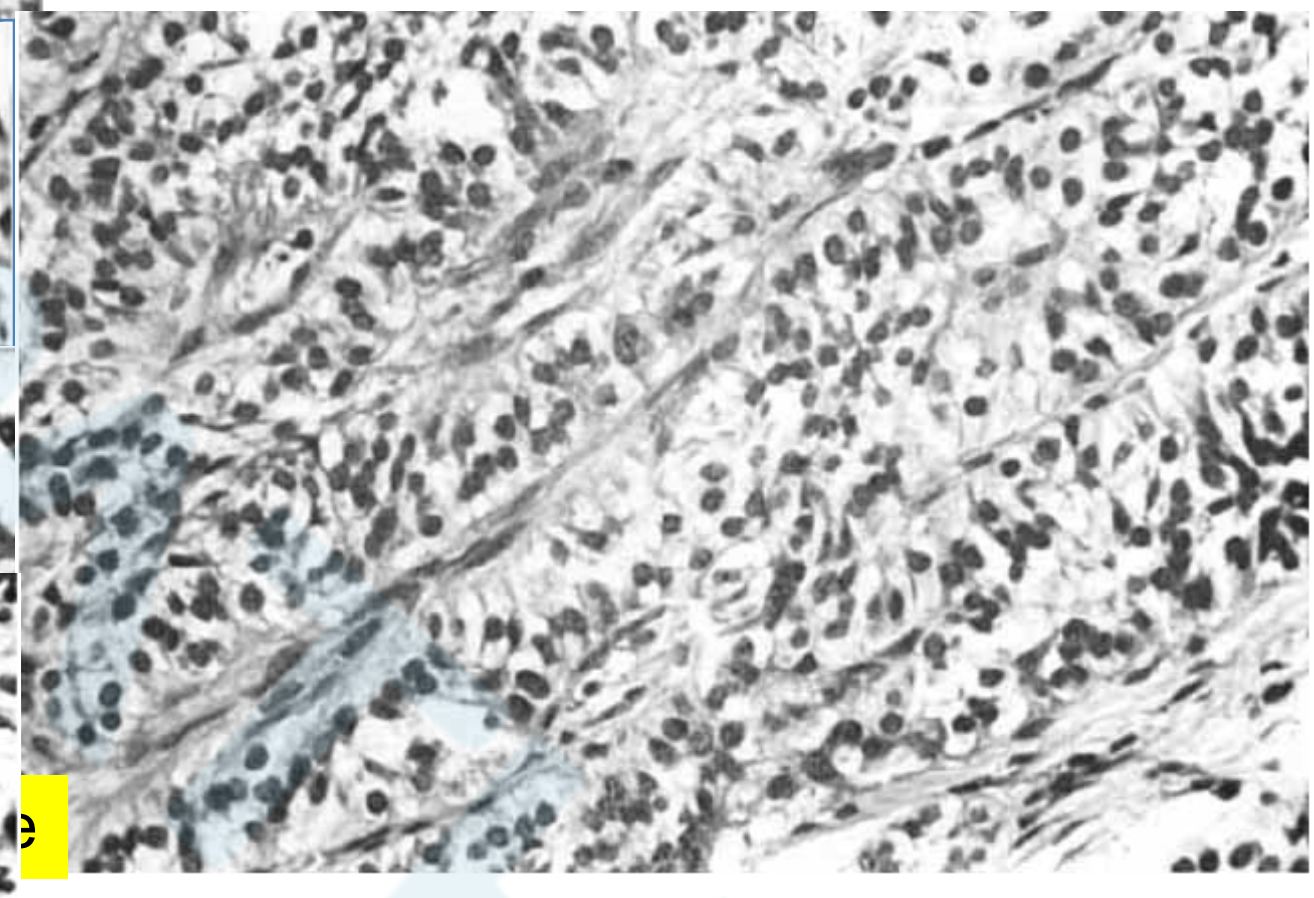








Mixed type



- Immunohistochemical Study

TABLE 1. Antibodies and Antisera Used

Antibodies/ Antisera	P/M (Clone)	Dilution	Source
Chromogranin A	M (LK2H10)	1:1	Ventana Medical System Inc., Tucson, AZ
Synaptophysin	M (snp88)	1:100	BioGenex Laboratories, San Ramon, CA
Serotonin	M (YC5)	1:50	Dako, Copenhagen, Denmark
Glicentin	P	1:2500	Milab, Malmo, Sweden
Somatostatin	P	1:500	Dako
β-catenin	M (15B8)	1:500	Santa Cruz Biotechnology Inc., Dallas, TX
p53	M (D07)	1:500	Dako
Ki67	M (MIB1)	1:100	Dako

P/M indicates polyclonal/monoclonal.

- Molecular Analysis

KRAS	exons 2 to 4, containing hotspot codons 12, 13, 61, 117,146
BRAF	exon 15,containing codon 600
PIK3CA	exons 9 to 20 containing codons 542, 545, and 1047
MSI	BAT25 and BAT26

RESULTS

TABLE 2. Clinico-pathologic Features of Digestive MANETs

						Ad. Component		Neuroendocrine Component					
Case	Age (y)	Sex	Symptoms	Site	Size (cm)	Type	Dysplasia	Size	Type*	Infiltration	Grade	NE hyp.	Follow-up (y)
1	55	M	Dyspepsia	Stomach	1.5	TA	HG	0.3	B	Muscularis mucosae	G1	Yes	AFD (27)
2	75	M	Cirrhosis	Duodenum	4	TVA	LG	1.6	A/C	Muscularis mucosae	G1	Yes	AFD (2)
3	77	F	Cirrhosis	Duodenum	1.8	TA	LG	0.1	A/C	Muscularis mucosae	G1	Yes	AFD (1)
4	32	F	Cholangitis	Duodenum	0.4	TA	LG	0.3	A	Lamina propria	G1	Yes	AFD (2)
5	54	M	Screening	Right colon	0.4	TA	LG	0.2	A	lamina propria	G1	No	AFD (9)
6	71	M	Screening	Right colon	3	TVA	HG	0.5	B	Muscularis mucosae	G3	No	AFD (1)
7	54	M	Screening	Sigma	4.5	TA	HG	0.4	A	Lamina propria	G2	No	AFD (4)
8	77	M	Diarrhea	Sigma	0.7	TVA	LG	0.3	A	Muscularis mucosae	G1	No	NA
9	54	M	Proctorrhagia	Rectum	2	TVA	LG	0.8	B	Muscularis mucosae	G1	Yes	AFD (24)
10	52	F	Proctorrhagia	Rectum	1	TA	HG	0.8	B	Sub-mucosa	G1	No	AFD (12)
11	60	F	Diarrhea	Rectum	0.3	TA	LG	0.2	B	Muscularis mucosae	G1	No	AFD (11)
12	53	M	Screening	Rectum	0.5	TA	LG	0.5	B	Muscularis mucosae	G2	No	AFD (12)
13	68	F	Dyspepsia	Stomach	0.4	TA	LG	†	†	†	G1	Yes	AFD (2)
14	65	M	Screening	Rectum	2.5	TVA	HG	†	†	†	G1	Yes	NA

TABLE 3. Immunohistochemical and Molecular Findings

	Ki67 (%)	p53 (%)		β-catenin			BAT 25-26		*KRAS*		*BRAF*		*PIK3CA*	
Case	NE	Gland	NE	Gland	NE	Additional Positivity	Gland	NE	Gland	NE	Gland	NE	Gland	NE
1	1	10	0	NA	NA	None	NA	NA	NA	NA	NA	NA	NA	NA
2	1	10	0	M	M	None	NA	NA	NA	NA	NA	NA	NA	NA
3	0.5	10	0	M	M	None	NA	NA	NA	NA	NA	NA	NA	NA
4	1	0	0	C and N	C and N	5HT	NA	NA	NA	NA	NA	NA	NA	NA
5	0.5	0	0	NA	NA	None	NA	NA	NA	NA	NA	NA	NA	NA
6	22	10	0	C and N	C and N	None	NA	NA	NA	NA	NA	NA	NA	NA
7	15	100	NA	N	NA	None	MSS	MSS	WT	WT	WT	WT	WT	WT
8	0.5	NA	NA	C and N	C and N	None	NA	NA	NA	NA	NA	NA	NA	NA
9	0.9	5	0	M	M	5HT	NA	NA	NA	NA	NA	NA	NA	NA
10	1	10	0	M	M	None	MSS	MSS	WT	WT	WT	WT	WT	WT
11	1	10	0	NA	NA	None	MSS	MSS	WT	WT	WT	WT	WT	WT
12	5	0	0	M	M	5HT, som	MSS	MSS	WT	WT	WT	WT	WT	WT
13	1	NA	NA	C and N	C and N	5HT	NA	NA	NA	NA	NA	NA	NA	NA
14	0.5	NA	NA	C and N	C and N	None	NA	NA	NA	NA	NA	NA	NA	NA

5HT indicates serotonin; C, cytoplasmic; Gland, glandular component; M, membrane; MSS, microsatellite stability; N, nuclear; NA, not available; NE, neuroendocrine component; som, somatostatin; WT, wild type.

- The average age :60 years (range: 32 to77 y)
- M>F
- Site:1/12 stomach, 3/12 duodenum, 8/12 colon
- The average size :1.7 cm(range: 0.3 to 4 cm)
- The glandular and NET components were intimately admixed and zone of transition between them were also detected
- 11/11 alive and free of disease, mean follow-up time ： 9 years (range: 1 to 27 y).
- No mutations in KRAS, BRAF, PIK3CA and no MSI in all cases

- Morphologic
  - Adenoma
    - Type
      - 8 tubular adenoma
      - 4 tubulo-villous adenoma
    - Degree of dysplasia
      - 8 LG
      - 4 HG
  - neuroendocrine component
    - Architectural pattern
      - 4 type A(small nests)
      - 6 type B (trabecular structures)
      - 2 A/C
    - Grade
      - 9 G1(ki67<3%)
      - 2 G2(3%<ki67<15%)
      - 1 G3(ki67:22%,3mitoses/10HPF)
    - Level of infiltration
      - 3 Lamina propria
      - 8 Muscularis mucosae
      - 1 Sub-mucosa

- The neuroendocrine component

➢ Localized in the deep central portion of the polyp

➢ The mean diameter : 0.5 cm (range: 0.1 to 1.6 cm)

➢ No mitotic figures were observed in the NET component of 11 cases, while 3/10HPF were found in 1 case

➢ No vascular invasion

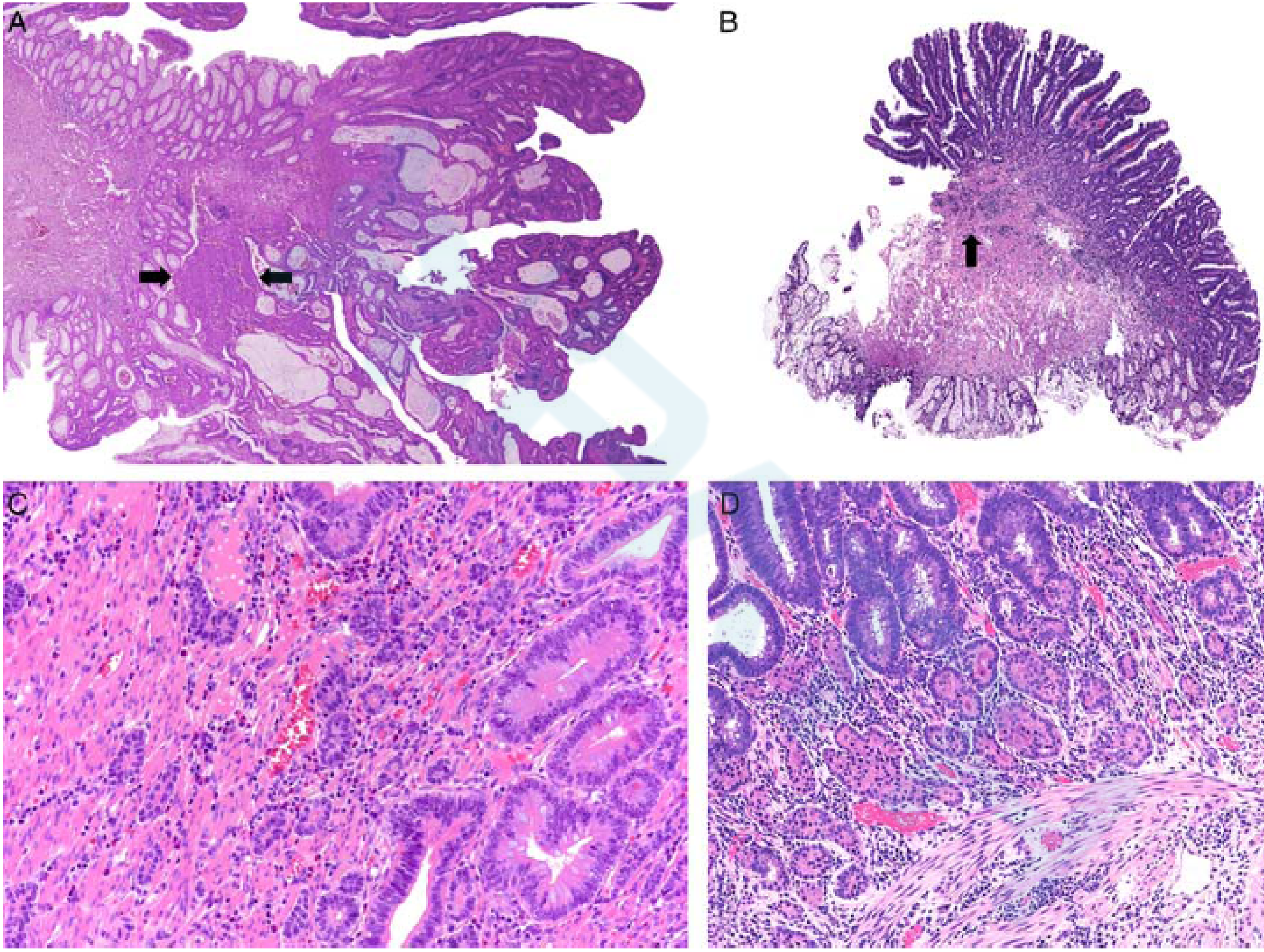


FIGURE 1. The neuroendocrine component of MANETs is localized in the deep central portion of the polyp (A, B, arrows) and consists of a proliferation of well-differentiated neuroendocrine cells forming small nests (C, D).

- The neuroendocrine component was positive for CgA and SYN in all cases
- CgA and SYN also showed the presence of neuroendocrine hyperplasia into the adjacent adenomatous component in 5 MANETs

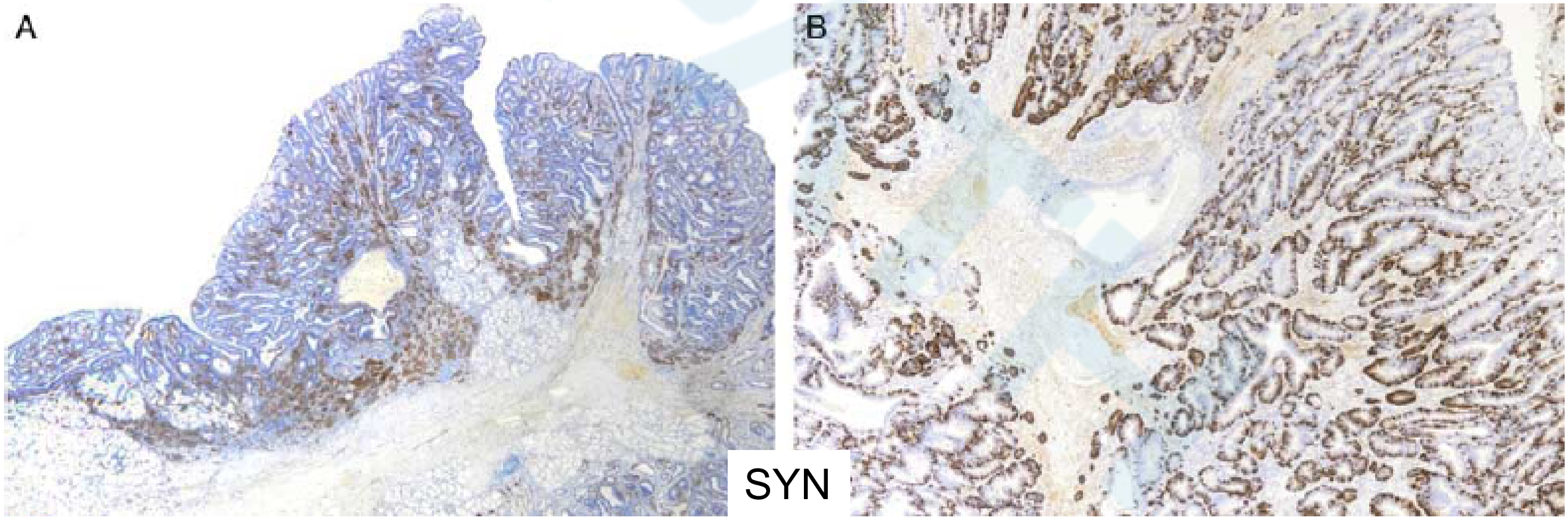
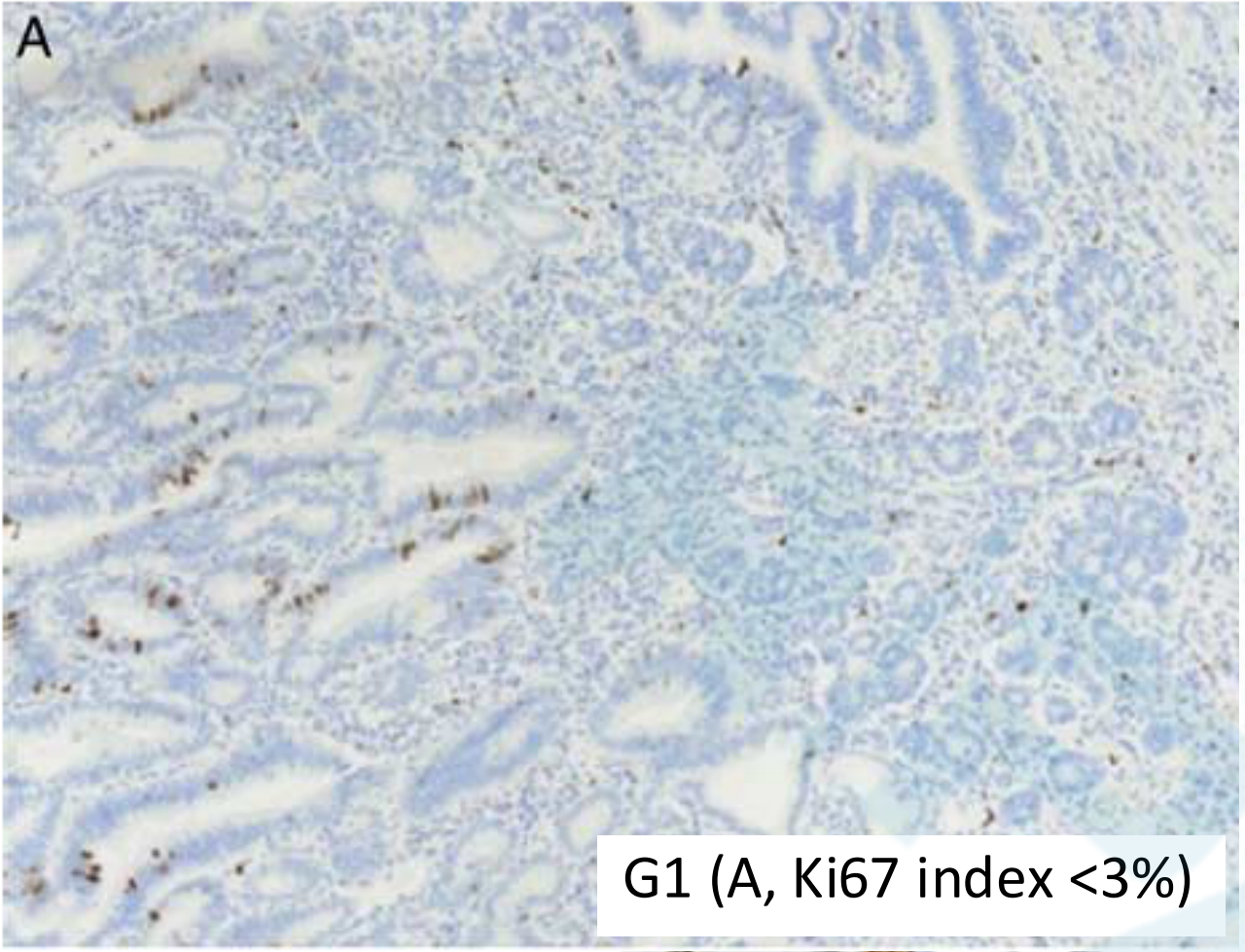
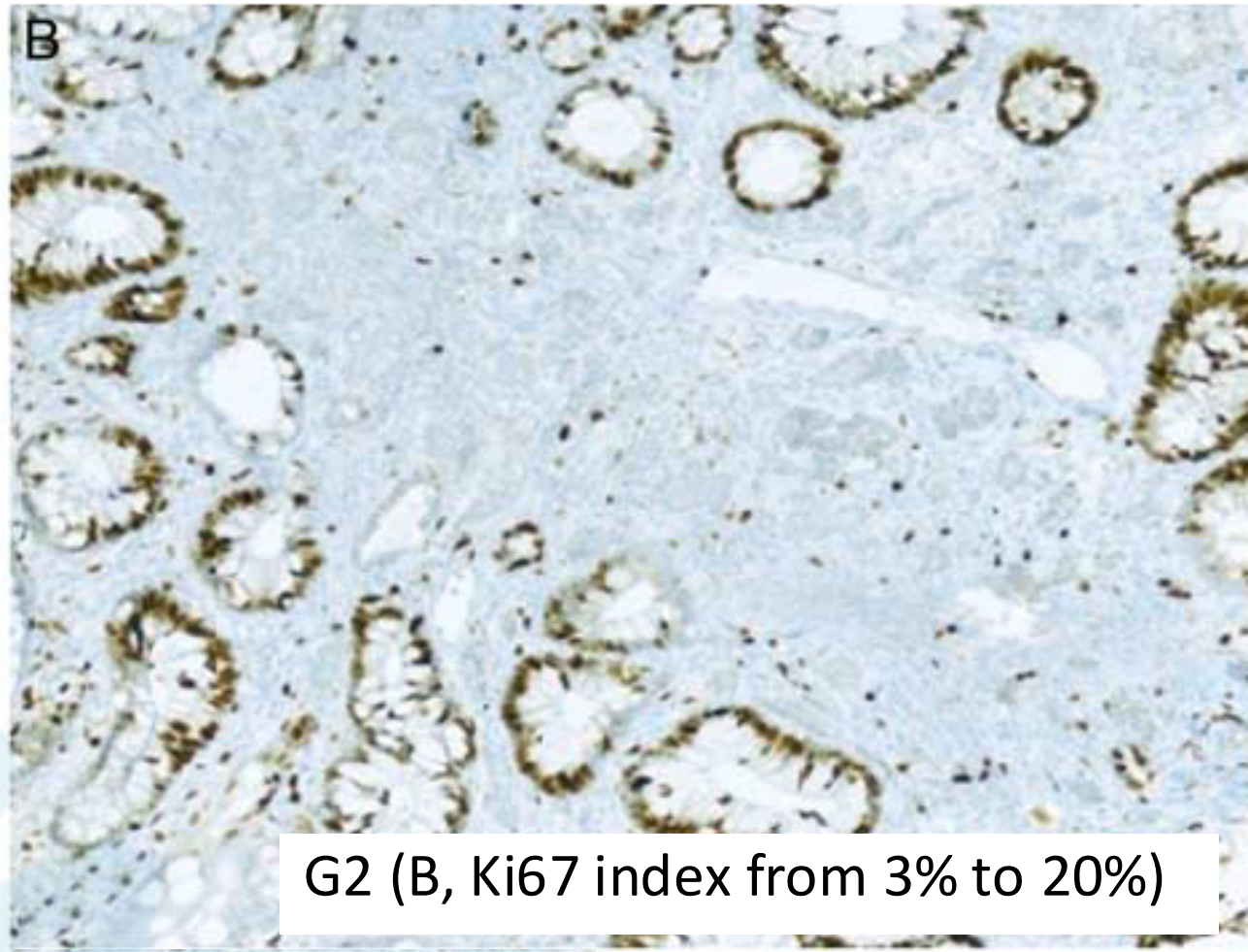


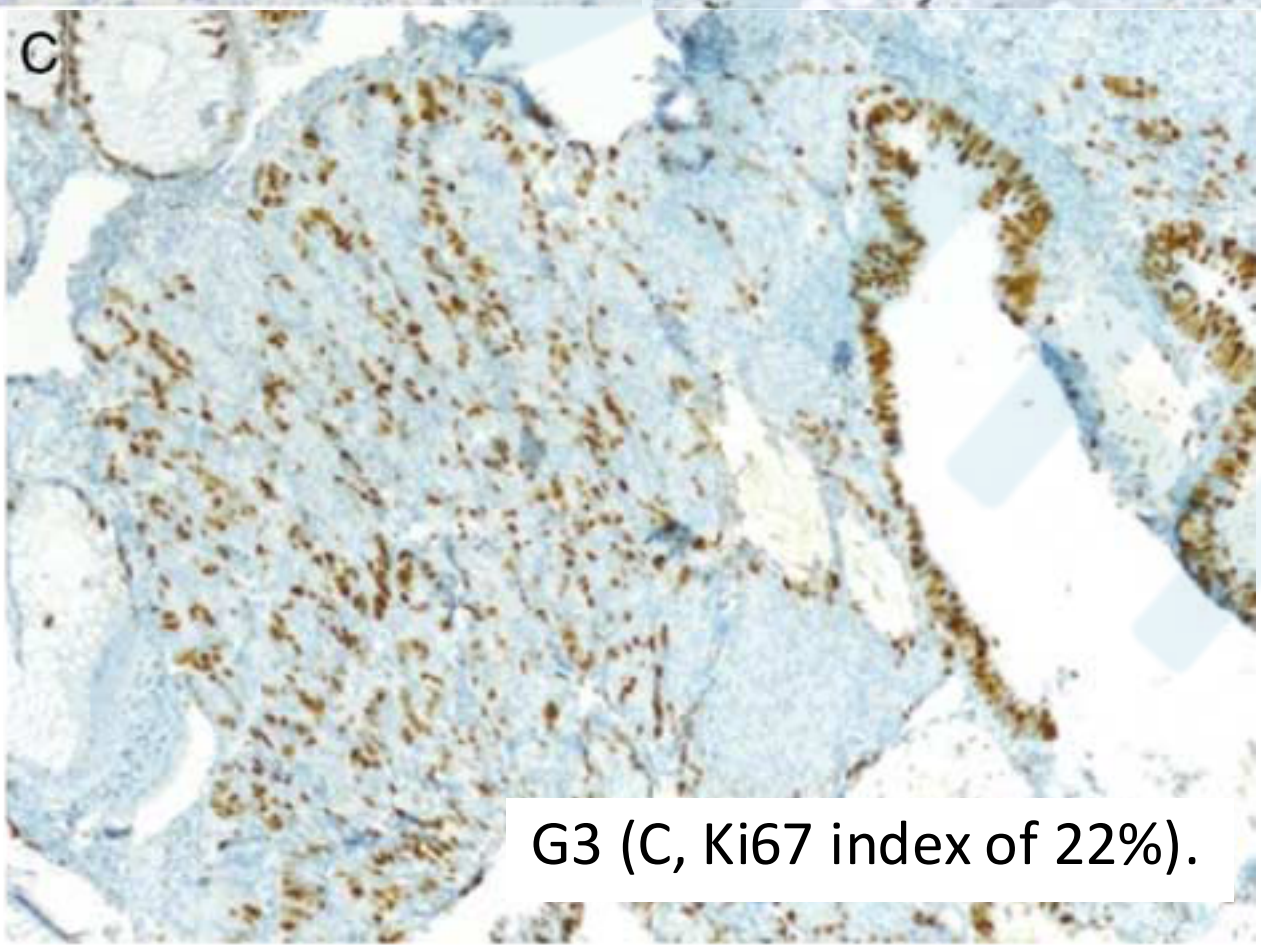
FIGURE 2. The neuroendocrine tumor component of MANETs is positive for general neuroendocrine markers including synaptophysin (A) and in several cases a neuroendocrine hyperplasia in the adenomatous component can be identified (B).



G1 (A, Ki67 index <3%)



G2 (B, Ki67 index from 3% to 20%)



G3 (C, Ki67 index of 22%).

- 0/11 P53 showed overexpression in neuroendocrine component
- β-catenin was performed in 9 cases and it showed variable cytoplasmic and nuclear positivity, in both tumor components

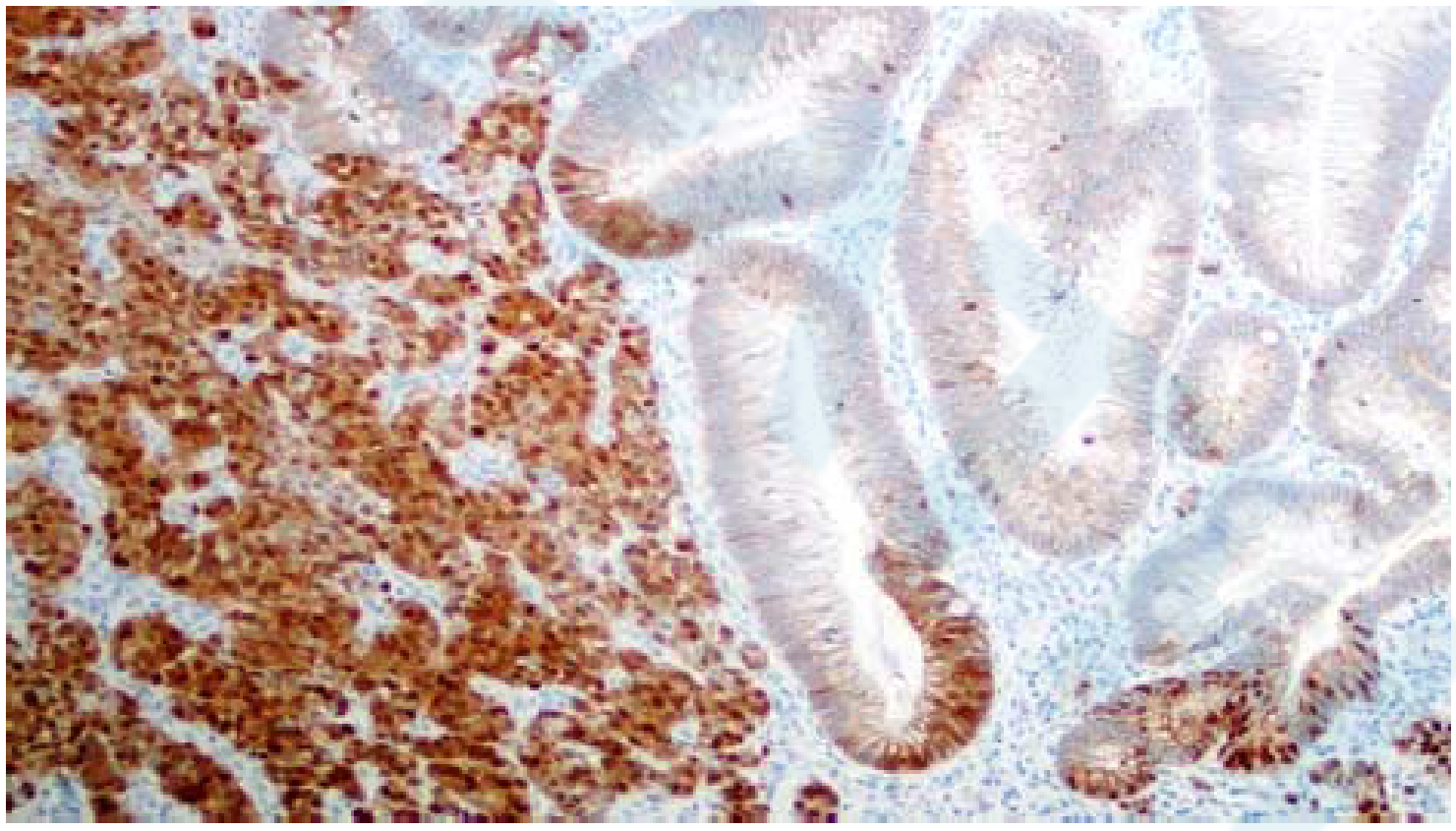
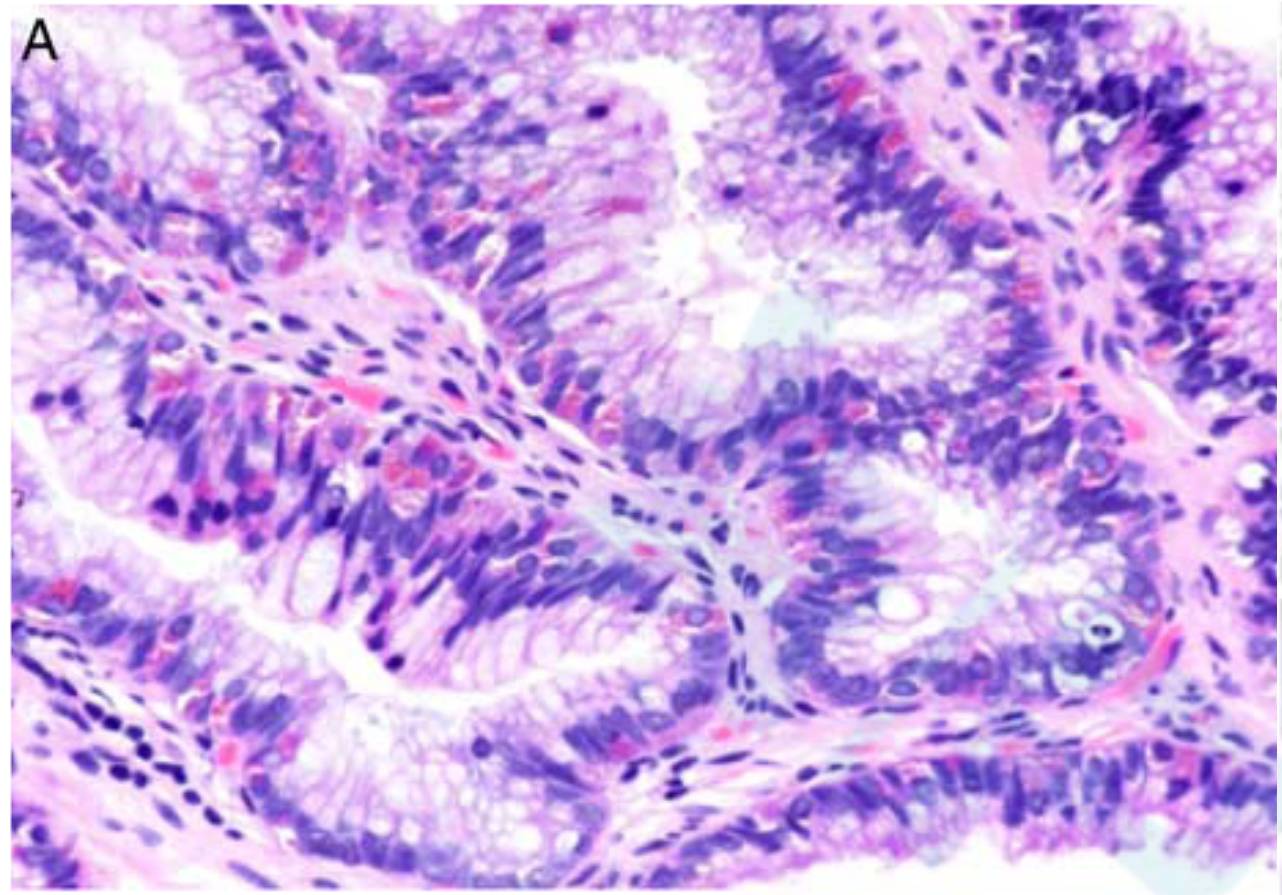
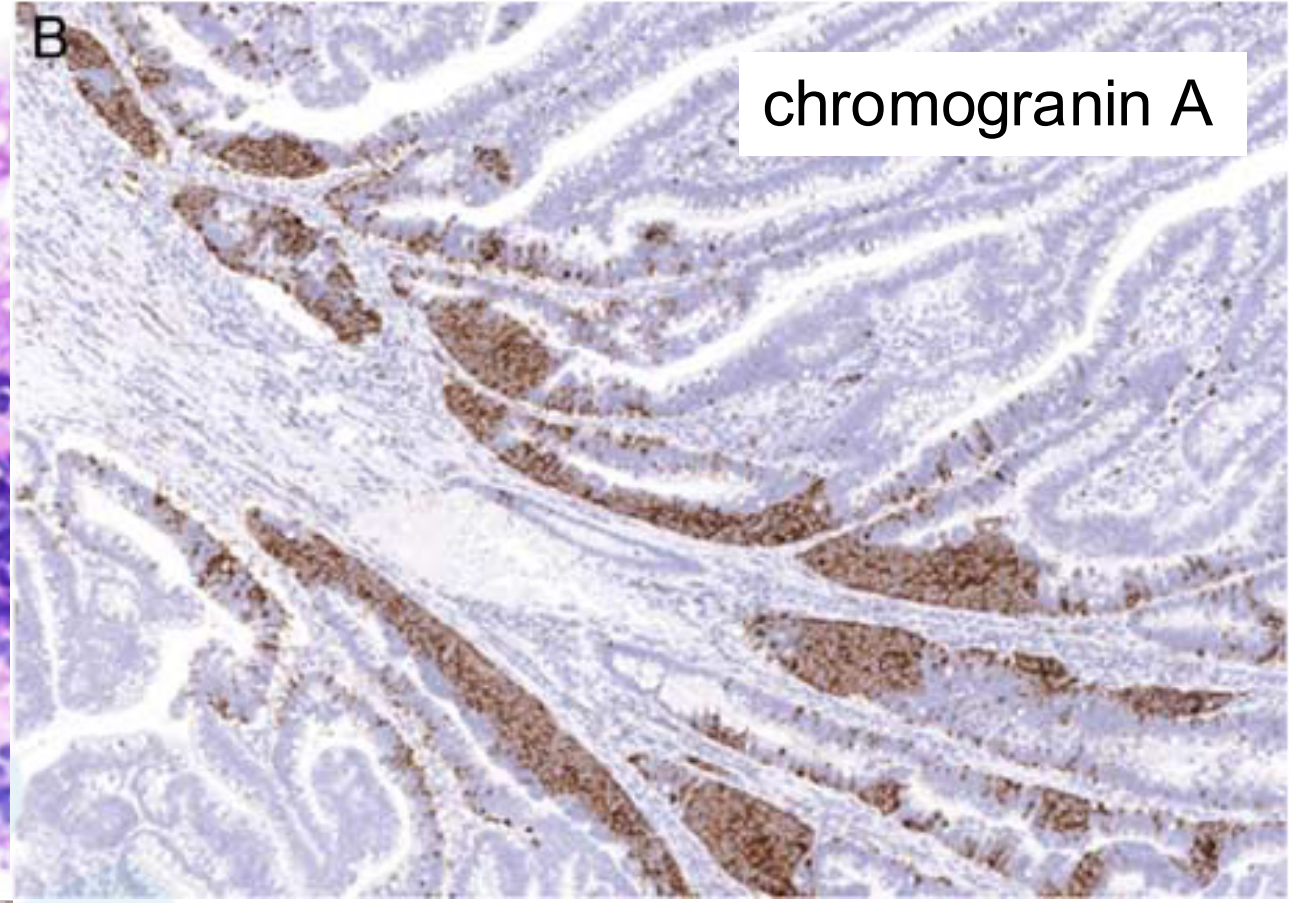


FIGURE 4. Colonic MANET showing strong and nuclear and cytoplasmic positivity for β-catenin in the neuroendocrine component, which was focal in the adenomatous one.





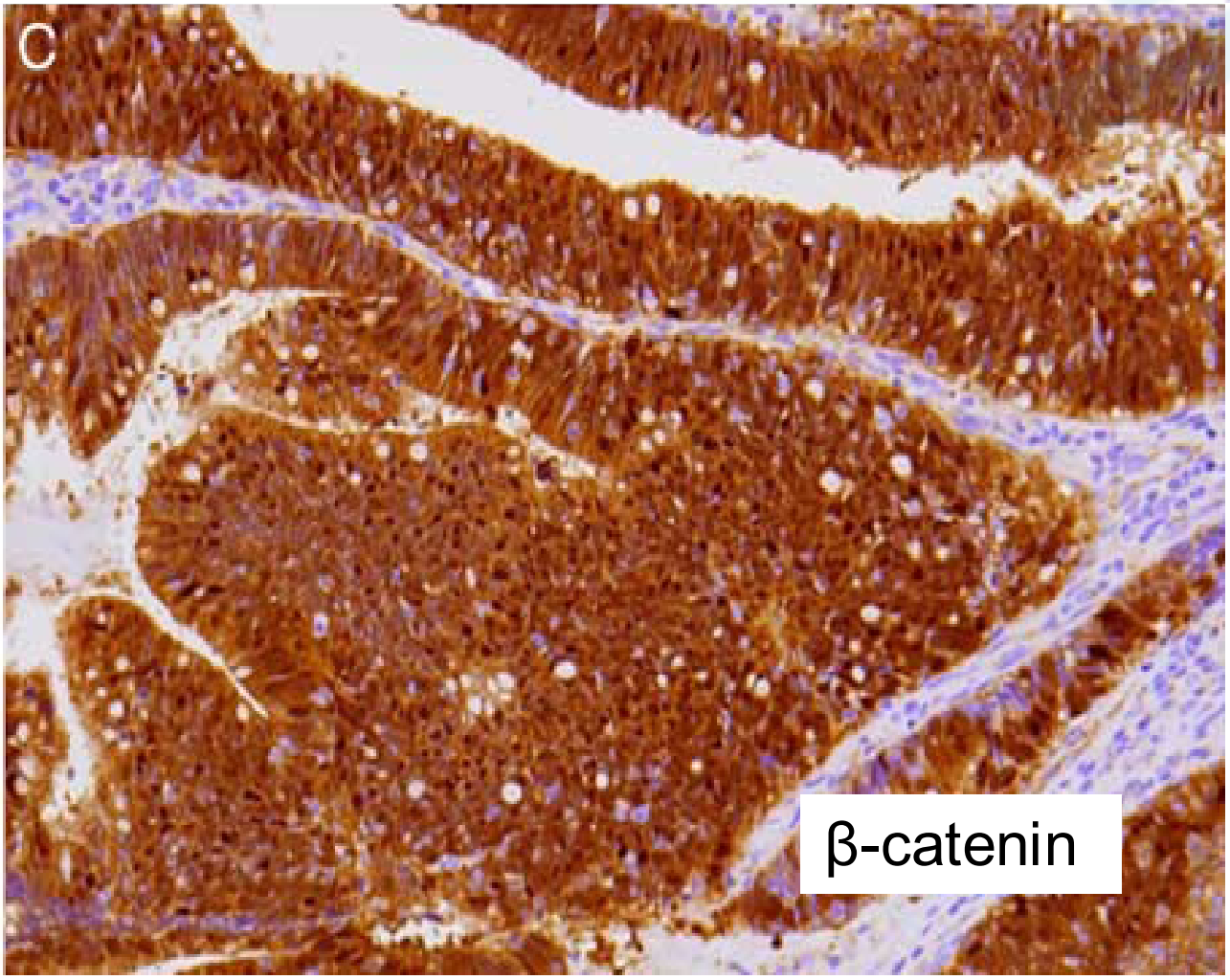


FIGURE 5. In 2 cases (cases # 13 and 14), the presence of numerous well differentiated cells with red and finely granular cytoplasm
was identified along the base of glandular adenomatous structures (A) and they were strongly positive for chromogranin A and reached >30% of the tumor mass (B). Both cases also showed a nuclear and cytoplasmic immunoreactivity for β-catenin (C).

DISCUSSION

1. Reviewed the literature on this topic and identified 71 cases including the 12 described in this paper

TABLE 4. Review of the Literature: Clinico-pathologic Features of MANETs

Case	References	Age (y)	Sex	Symptoms	Site	Size (cm)	Adenoma Type	Adenoma Dysplasia
1	Ito et al[13]	54	M	None	Stomach	2	TA	LG
2	Harada et al[14]	72	M	None	Stomach	1	NA	NA
3	De Marco et al[15]	76	M	Dysphagy	Stomach	1	TA	LG
4	Coyne and O'Connor[16]	68	F	Abdominal discomfort	Stomach	1.1	TA	LG
5	Lee et al[17]	64	M	None	Stomach	3.8	TA	NA
6	Lee et al[17]	63	M	Abdominal discomfort	Stomach	4	TA	NA
7	Lee et al[17]	52	M	None	Stomach	1.8	TA	NA
8	Lee et al[17]	65	M	Dyspepsia	Stomach	4.1	TVA	HG
9	Case #1	55	M	Dyspepsia	Stomach	1.5	TA	HG
10	July et al[18]	72	F	FAP	Duodenum	2	TA	LG
11	July et al[18]	72	M	Screening	Duodenum	1.2	TA	HG
12	July et al[18]	61	M	Screening	Duodenum	0.5	TA	LG
13	Case #2	75	M	Cirrhosis	Duodenum	4	TVA	LG
14	Case #3	77	F	Cirrhosis	Duodenum	1.8	TA	LG
15	Case #4	32	F	Cholangitis	Duodenum	0.4	TA	LG
16	Varghese et al[19]	76	F	Bleeding	Ileum	4	TA	LG
17	Moyana et al[20]	68	F	Abdominal discomfort	Cecum	1.5	NA	NA
18	Pulitzer et al[21]	77	M	None	Cecum	1.3	TA	LG
19	Pulitzer et al[21]	77	F	None	Cecum	2	TVA	HG
20	Pulitzer et al[21]	77	F	None	Cecum	1	TA	HG
21	Lin et al[22]	66	F	Screening	Cecum	1.5	TA	HG
22	Lyda and Fenoglio-Preiser[23]	80	M	Bleeding	Right colon	3	TVA	HG
23	Lin et al[22]	69	F	Screening	Right colon	5.5	TA	LG
24	Case #5	54	M	Screening	Right colon	0.4	TA	LG
25	Case #6	71	M	Screening	Right colon	3	TVA	HG
26	Salaria et al[24]	55	M	Bleeding	Right colon	5.3	TA	LG
27	Salaria et al[24]	28	F	Bleeding	Right colon	3.4	TVA	HG
28	Salaria et al[24]	82	M	Screening	Right colon	1.5	TVA	LG
29	Salaria et al[24]	60	F	Bleeding	Right colon	0.7	TVA	HG
30	Salaria et al[24]	54	F	Screening	Transverse colon	1.4	TA	HG
31	Pulitzer et al[21]	62	F	None	Left colon	5	TA	LG
32	Lin et al[22]	56	M	Colonic cancer	Left colon	2.5	TVA	HG
33	Bazerbachi et al[25]	72	M	NA	Left colon	2	TVA	LG
34	Case #7	54	M	Screening	Left colon	4.5	TA	HG
35	Case #8	77	M	Diarrhea	Left colon	0.7	TVA	LG
36	Salaria et al[24]	81	F	Diarrhea	Left colon	1.2	TVA	HG
37	Salaria et al[24]	55	M	Screening	Left colon	1.2	TA	LG
38	Salaria et al[24]	48	M	NA	Left colon	1.5	TVA	LG
39	Salaria et al[24]	62	M	Screening	Left colon	2.5	TVA	LG
40	Salaria et al[24]	51	F	Screening	Left colon	3	TVA	LG
41	Mori et al[26]	75	M	Bleeding	Rectum	1.3	TVA	LG
42	Moyana et al[20]	75	F	Constipation	Rectum	2	VA	NA
43	Lyda and Fenoglio-Preiser[23]	37	M	Diarrhea	Rectum	1	TA	LG
44	Lin et al[22]	51	M	Screening	Rectum	2	TVA	HG
45	Lin et al[22]	59	M	FAP	Rectum	1.3	TA	HG
46	Hui[27]	45	F	Diarrhea	Rectum	1.5	TVA	HG
47	Case #9	54	M	Proctorrhagia	Rectum	2	TVA	LG
48	Case #10	52	F	Proctorrhagia	Rectum	1	TA	HG
49	Case #11	60	F	Diarrhea	Rectum	0.3	TA	LG
50	Case #12	53	M	Screening	Rectum	0.5	TA	LG
51	Salaria et al[24]	72	F	None	NA	1.5	TVA	LG
52-71	Estrella et al‡[28]							

*According to Soga and Tazawa.[4]

†All patients were AFD.

‡The cases published by Estrella et al[28] are not included because, from the paper, the clinico-pathologic characteristics of patients with MANETs cannot be isolated from cases in which a malignant component is also presents.

AFD indicates alive free of disease; AWD, alive with disease; DOC, died of other cause; F, female; FAP, familial adenomatous polyp; FUP, follow-up; HG, high grade; L, lost at follow-up; LG, low grade; M, male; METS, metastases; MTS, metastases; NA, not available; NE hyperpl, neuroendocrine hyperplasia; NET, neuroendocrine tumor; TA, tubular adenoma; TVA, tubulo-villous adenoma; VA, villous adenoma.

TABLE 4. *(Continued)*

NET Size	NET Type*	NET Infiltration	NET Grading	Associated NE hyperpl.	MTS	FUP (y)	Nuclear β-catenin
0.4	A	Muscularis mucosae	NA	No	NA	AFD (19)	NA
NA	A	Lamina propria	NA	NA	No	NA	NA
0.2	A	Muscularis mucosae	G1	No	NA	AFD (10)	NA
NA	A	Submucosa	G1	Yes	NA	NA	NA
NA	A	Muscularis mucosae	NA	Yes	NA	AFD (2)	NA
NA	A	Lamina propria	NA	Yes	NA	AFD (2)	NA
NA	A	Muscularis mucosae	NA	Yes	No	AFD (2)	NA
NA	A	Submucosa	NA	Yes	NA	AFD (12)	NA
0.3	B	Muscularis mucosae	G1	Yes	No	AFD (27)	NA
NA	A	Lamina propria		No	No	AWD (2)	NA
0.2	A	Lamina propria	G1	No	No	L	NA
0.2	A	Lamina propria	G1	No	No	L	NA
1.6	A/C	Muscularis mucosae	G1	Yes	No	AFD (2)	No
0.1	A/C	Muscularis mucosae	G1	Yes	No	AFD (1)	No
0.3	A	Lamina propria	G1	Yes	No	AFD (2)	Yes
NA	A	Subserosa	NA	No	Yes	NA	NA
NA	NA	NA	NA	No	No	AFD (2)	NA
NA	A/C	Lamina propria	G1	No	No	AFD (10)	NA
NA	A/C	Lamina propria	G1	No	No	AFD (1)	NA
0.5	A/C	Lamina propria	G1	No	No	AFD (3)	NA
0.1	NA	Lamina propria	G1	No	NA	AWD (0.5)	NA
NA	A/C	Lamina propria	na	No	No	DOC (2)	NA
0.1	NA	Submucosa	G1	No	NEC	AWD (1.5)	NA
0.2	A	Lamina propria	G1	No	No	AFD (9)	NA
0.5	B	Muscularis mucosae	G3	No	No	AFD (1)	Yes
0.2	NA	NA	G1	NA	NA	†	Yes
0.7	NA	NA	G1	NA	NA	†	Yes
0.5	NA	NA	G1	NA	NA	†	Yes
0.3	NA	NA	G1	NA	NA	†	Yes
0.1	NA	NA	NA	NA	NA	†	NA
1.5	A/C	Lamina propria	G1	Yes	No	AFD (1)	NA
0.1	NA	Lamina propria	G1	NO		AWD (0.5)	NA
0.3	A	Muscularis propria	NA	NA		AFD (1)	NA
0.4	A	Lamina propria	G2	No	No	AFD (4)	NA
0.3	A	Muscularis mucosae	G1	No	No	L	Yes
0.3	NA	NA	G1	NA	NA	†	NA
0.7	NA	NA	NA	NA	NA	†	NA
0.4	NA	NA	G1	NA	NA	†	Yes
0.4	NA	NA	NA	NA	NA	†	NA
0.2	NA	NA	G1	NA	NA	†	Yes
0.6	NA	Lamina propria	NA	NA	No	AFD (1)	NA
NA	NA	NA	NA	No	No	AFD (1)	NA
NA	A/C	Muscularis mucosae	NA	No	No	L	NA
0.1	NA	Lamina propria	G1	No		AWD (1)	NA
0.1	NA	Lamina propria	G1	No		AWD (0.2)	NA
NA	A	NA	NA	NA		L	NA
0.8	B	Muscularis mucosae	G1	Yes	No	AFD (24)	No
0.8	B	Submucosa	G1	No	No	AFD (12)	No
0.2	B	Muscularis mucosae	G1	No	No	AFD (11)	NA
0.5	B	Muscularis mucosae	G2	No	No	AFD (12)	No
0.5	NA	NA	NA	NA	NA	†	NA

- More frequent in males (male-to-female ratio:1.4)
- The average age at the time of diagnosis was 63 years
- They were described all along the gut, including stomach, duodenum, ileum, right colon,transverse colon, left colon, and rectum
- 47/52 (90)% was NET G1 , 4/52(8%) was G2 , 1/52(2%) was G3(well-differentiated neuroendocrine tumor with high-grade proliferative features)
- Neuroendocrine hyperplasia was reported in the adjacent adenoma in 11/35 (31%) cases
- The adenoma was tubular type(56%), tubulo-villous(41%) , villous(3%),LG in 60% and HG in 40%

- MANETs can show a wide spectrum of different combinations including different types of adenomatous component, with either low-grade or high-grade dysplasia,and different NET types, ranging from NET G1 to NET G3
- All patients with available follow-up data were alive (mean follow-up time: 6 y, range: 0.5 to 27 y), While 1 patient died of disease

(The deceased patient presented metastatic dissemination of NEC, which was not present in the tumor resection diagnosed as MANET.)

2.The available data strongly support that MANET is an indolent disease, which needs to be separated from other MiNENs with a NEC component(MANECs) as the latter represents aggressive neoplasms with an average overall survival rate of 13.2 months

- Considering the indolent nature of the lesion ,the therapeutic approach (polypectomy) for MANET would be the same as pure adenoma

3. None of our cases showed KRAS mutations, neither in the adenoma nor in the NET component. The adenoma and NET components of MANET develop through a different pathway, compared to pure adenoma or NET

- The NET component of MANETs showed a more frequently β-catenin nuclear immunoreactivity than pure NETs. This supports the clonal origin of both components from a multipotent stem cell, which undergoes a divergent differentiation. And it suggests that the neuroendocrine proliferation/hyperplasia may be considered as a preneoplastic or an in situ clonal proliferation.

4.The results of the present study, together with the review of the previously reported cases,support the concept that digestive MiNENs are a heterogenous group of proliferations, encompassing a spectrum of diseases with different morphology, clinical behavior and biology.

- Low-grade MiNENs (MANETs), rare indolent lesions, association of an adenoma and a well-differentiated NET
- High-grade MiNENs(MANECs) , aggressive neoplasms , association of a poorly differentiated NEC with a non-neuroendocrine epithelial component, most frequently an adenocarcinoma
- Intermediate grade neoplasms, composed of adenocarcinoma and NET

TABLE 5. Classification and of MINENs of the Digestive System

MINENs
Low-grade MiNEN
Gut
MANET
Intermediate-grade MiNEN
Gut
Mixed adenocarcinoma-NET
High-grade MiNEN
Gut
Mixed adenoma/adenocarcinoma-NEC
Mixed squamous cell carcinoma-NEC
Pancreas
Mixed ductal adenocarcinoma-NEC*
Mixed acinar cell carcinoma-NEC*
Mixed acinar-ductal carcinoma-NEC
Biliary system
Mixed adenocarcinoma-NEC
Liver
Mixed hepatocellular carcinoma-NEC
Mixed cholangiocarcinoma-NEC

*The rare cases in which the neuroendocrine component is represented by a NET G2 should be included in this group.


Thank
You!