Original paper

Prevalence, distribution, and prognostic significance of morphological variants of neuroendocrine tumors of the gastrointestinal tract – a multicenter study

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The incidence and prevalence of neuroendocrine neoplasms (NENs) in many organs are increasing. Although such NENs have similar grades, they may exhibit quite different behaviors. In this multicenter study, we aimed to investigate the prevalence and distribution of different morphological NEN variants in the non-pancreatic gastrointestinal (GI) tract and determine whether they can guide prognosis prediction.

Two hundred and fifty-six patients diagnosed with NENs originating from the GI tract from 7 different centers were included in the study. In 89 (36.6%) cases, different morphological variants were detected.

When the variants were grouped according to their aggressiveness as described in the literature, a statistically significant relationship between aggressiveness and the variables organ and age was found ($p < 0.05$). The oncocytic variant was found to metastasize more than the other aggressive types (42.9%). The paraganglioma-like variant was found to have a smaller size, lower proliferation index, and a more benign clinical course.

This study demonstrated that well-differentiated GI neuroendocrine tumors (GI-NETs) have considerable morphological diversity. Generally, case reports of rare morphological variants of GI-NETs are available in the literature. We believe that our study contributes to a better understanding of the prevalence, localization, and significance of morphological variations in GI-NETs.

Key words: neuroendocrine tumor, gastrointestinal tract, morphologic variants.

Introduction

Neuroendocrine neoplasms (NENs) are heterogeneous tumors with a unique histology and immunophenotype. They can be found in many organs where endocrine cells are present, and their incidence has been increasing in recent years [1]. The term "carcinoid" was first used in 1907 to describe these tumors, which were considered less benign than adenomas but not as aggressive as carcinomas [2]. In 1963, they were classified according to embryological developmental regions, and in 1986, according to histological features [3, 4]. In 1995, Capella *et al*. proposed the term neuroendocrine tumor (NET) instead of carcinoid [5]. In 2010, the World Health Organization (WHO) classified NETs into Grades 1, 2, and 3 according to the Ki-67 proliferation index and mitotic count [6]. The framework for the current classification was completed in 2017, and the current version was included in the WHO's digestive system in 2019 [1, 7]. Currently, NENs are divided into NETs and neuroendocrine carcinomas (NECs) based on the mitotic count, Ki-67 proliferation index, and histological differentiation [1].

Current classification studies that have been ongoing for years have the common aim of predicting disease prognosis. However, NETs are known to exhibit different behaviors despite having the same grade [8]. It has been suggested that some special variants of pancreatic neuroendocrine tumors (Pan-NETs) affect tumor behavior and can be used as prognostic markers [8–11]. Only isolated case reports and small series on special variants of non-pancreatic gastrointestinal (GI) NETs can be found in the literature [8–27].

In this multicenter study, we aimed to investigate the frequency of rare morphological NET variants in the GI tract, their distribution in various organs, their clinicopathological features, their relationship with grade, and whether they serve as a guiding factor in prognosis prediction. In doing so, a comprehensive series on the morphological analysis of NETs regarding rare morphological variants in the GI tract will be obtained.

Material and methods

Between January 2018 and January 2023, 256 patients diagnosed with NETs and NECs originating from the esophagus, stomach, small bowel, large bowel, or appendix from 7 different centers were included in our study. These included 94 cases from Center 1, 37 cases from Center 2, 66 cases from Center 3, 29 cases from Center 4, and 10 cases each from Centers 5, 6, and 7.

Information on age, gender, biopsy method, and localization site was obtained from the records in the hospitals' information systems. Hematoxylin and eosin (HE)-stained slides in the pathology archives and all immunohistochemically stained slides helpful in diagnosing and grading the cases were re-evaluated. Mitosis was counted in 50 high power fields (HPFs; 400×) and proportioned to 10 HPFs (2 mm^2). For the Ki-67 proliferation index, all slides were scanned and a "hotspot" area was determined. At least 500 tumor cells were counted in the hotspot area, and the percentage of Ki-67 was recorded. In the event of any discordance between Ki-67 and mitotic count, the larger value was used for grading [9, 10]. Grading was performed according to Ki-67, mitotic count, and histological differentiation [1]. The expression status of neuroendocrine markers helpful for diagnosis was recorded.

In classical morphology, the cells were relatively monotonous and round, with a moderate amount of cytoplasm. Coarse, granular "salt-and-pepper" chromatin was characteristic in centrally located nuclei (Fig. 1). The presence of nests, ribbons, rosettes, or trabeculae separated by a thin fibrovascular stroma represented the classical morphology [8] (Fig. 2).

All slides were evaluated for any diversion from the classical NET patterns. Morphological variants were identified according to the detailed description of the study describing Pan-NET morphological variants [8] (Fig. 3). In tumors with more than 1 variant, the most dominant component was taken as the basis. Hepatoid and paraganglioma-like variants were confirmed by immunohistochemical studies (Hep-Par1, S100, and pancytokeratin). Tumors differing from the classical morphology were recorded regardless of their proportions. Some tumor sections showed foci with areas containing pleomorphic cells, but the cells were few in number. These areas were considered to be endocrine atypia expected in endocrine tumors (Fig. 4). The site of metastasis was recorded in metastatic cases.

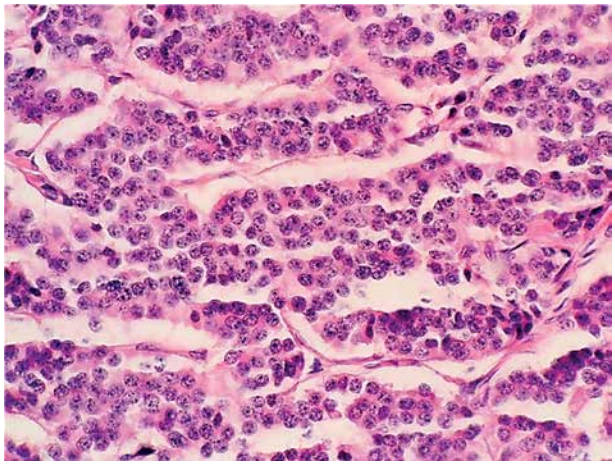

Fig. 1. Tumor cells, which consist of monotonous cells with centrally located nuclei, have "salt-and-pepper" chromatin (HE 200×)

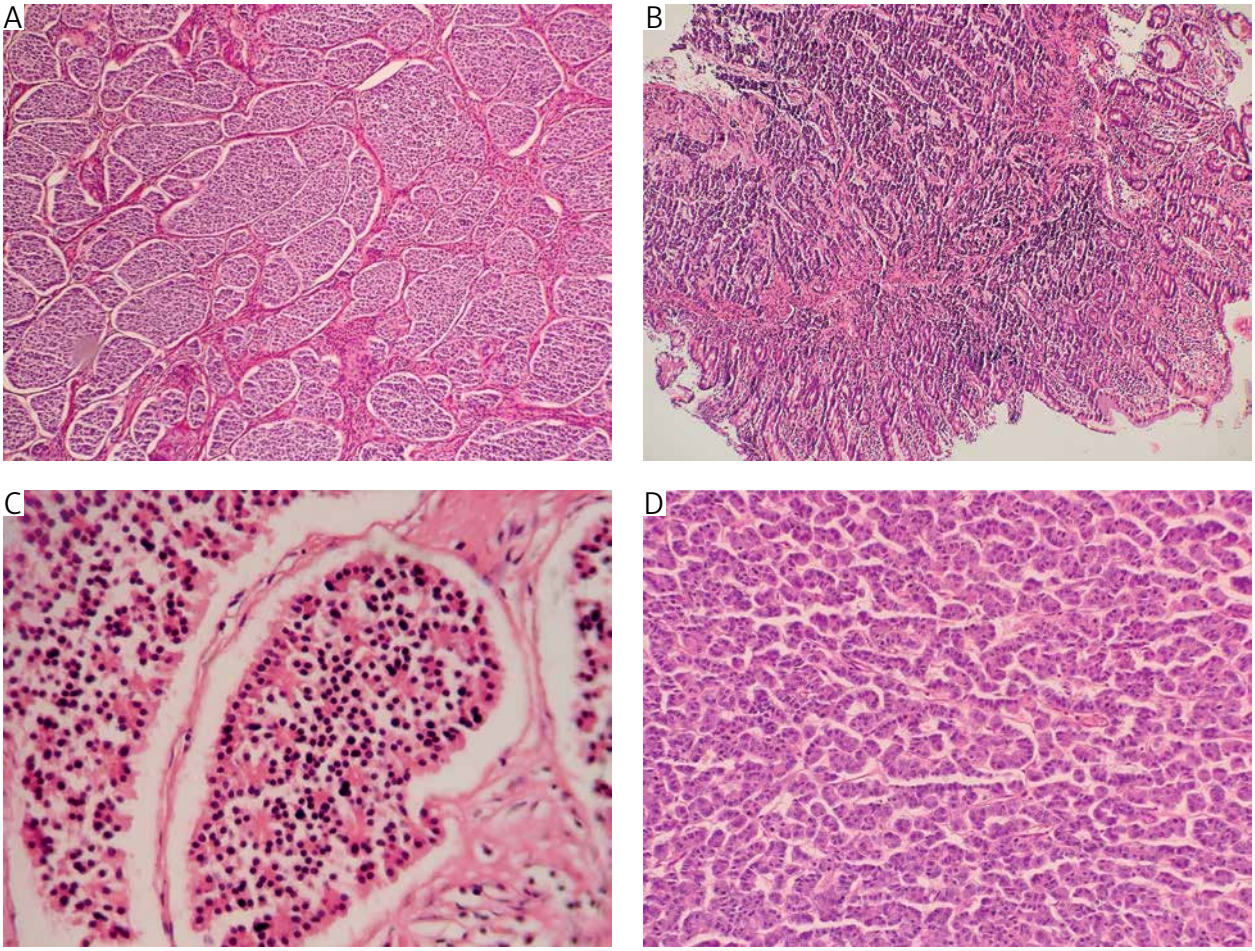

Fig. 2. Neuroendocrine tumors in classical morphology. A) Cells grow in nests (HE 40×). B) Cells grow in trabeculae (HE 40×). C) Cells grow in rosettes (HE 200×). D) Cells grow in ribbons (HE 40×)

The presence of statistically significant differences between classical NETs and NETs with morphological variants was investigated in terms of gender, age, organ location, tumor size, degree of invasion, grade, and mitosis, as well as prognostic factors including Ki-67 and the presence of metastasis. Demographic data of less frequent NECs were recorded.

The morphological variants were categorized into a less aggressive group, an indeterminate behavior group, and a more aggressive group, as described in a study on the pancreas [8]. The paraganglioma-like variant was included in the less aggressive group, while mammary tubulo-lobular carcinoma-like (MTLC-like) and pseudoglandular/tubular variants were included in the indeterminate behavior group. Discohesive, plasmacytoid, hepatoid, oncocytic, and lipid-rich variants were included in the more aggressive group. After this grouping, the appendiceal tubular variant was separated from the pseudoglandular variant and included in the less aggressive group since it included cases with behavioral characteristics that were reported to be better than those of the overall cohort, following which re-evaluations were performed.

The sections prepared from the paraffin blocks of suspected cases were stained with immunohistochemical antibodies of Hep-Par1 (OCHIE5 clone, 1 : 100, Zeta) and pancytokeratin (AE3 clone, 1 : 100, Cell Marque; S100: 4C4.9 clone, 1/150, Zeta) to determine and confirm the presence of the hepatoid and paraganglioma-like variants.

Statistical analysis

The Mann-Whitney *U* test and/or Student's *t*-test were used to compare the variables obtained by measurement between independent groups. The χ^2 and Fisher's exact tests were used to examine the relationships or differences between groups in terms of categorical variables. Regarding descriptive statistics, percentages and frequency distributions were calculated for categorical variables, while mean and standard deviation and median (minimum–maximum) values were calculated for continuous variables. Univariate logistic regression analysis was used to evaluate the risk factors for metastasis, and the results were summarized using odds ratios and 95% confidence intervals. IBM SPSS Statistics for Windows, Version 26.0 (IBM SPSS Statistics for Windows, Ver-

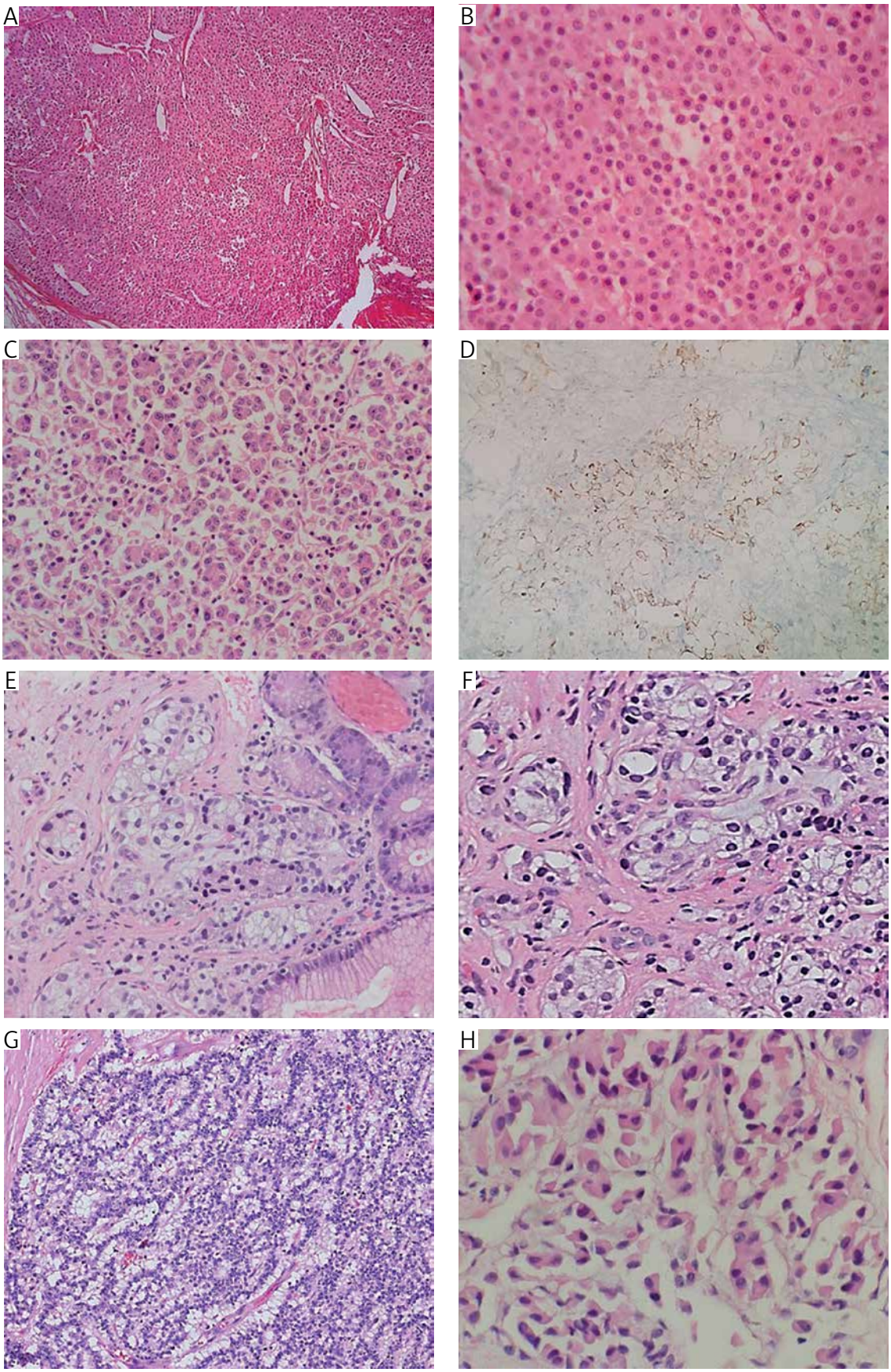

Fig. 3. A, B) Oncocytic variant often had sheet-like configuration (A – HE 40×). Oncocytic cells were characterized by abundant eosinophilic cytoplasm, smooth nuclear membrane, and prominent eccentric nucleoli (B – HE 200×). C, D) Hepatoid variant: polygonal cells with enlarged cytoplasm, central nuclei, and prominent nucleoli (C – HE 100×). Hepatoid variant cells show cytoplasmic granular staining at varying rates with Hep-Par1 (D – Hep-Par1 100×). E, F) Lipid-rich variant neuroendocrine tumors (NET) (HE 100×). At high magnification, it is observed that the cells have hyperchromatic, eccentrically located nuclei and their cytoplasm has a foamy appearance (HE 200×). G) Discohesive variant: pseudo-papillae lined by discohesive cells mimicking solid pseudopapillary neoplasm (HE 40×). H) Plasmacytoid/rhabdoid variant was characterized by eccentric nucleus, relatively homogeneous eosinophilic cytoplasm, and discohesive pattern of growth (HE 400×)

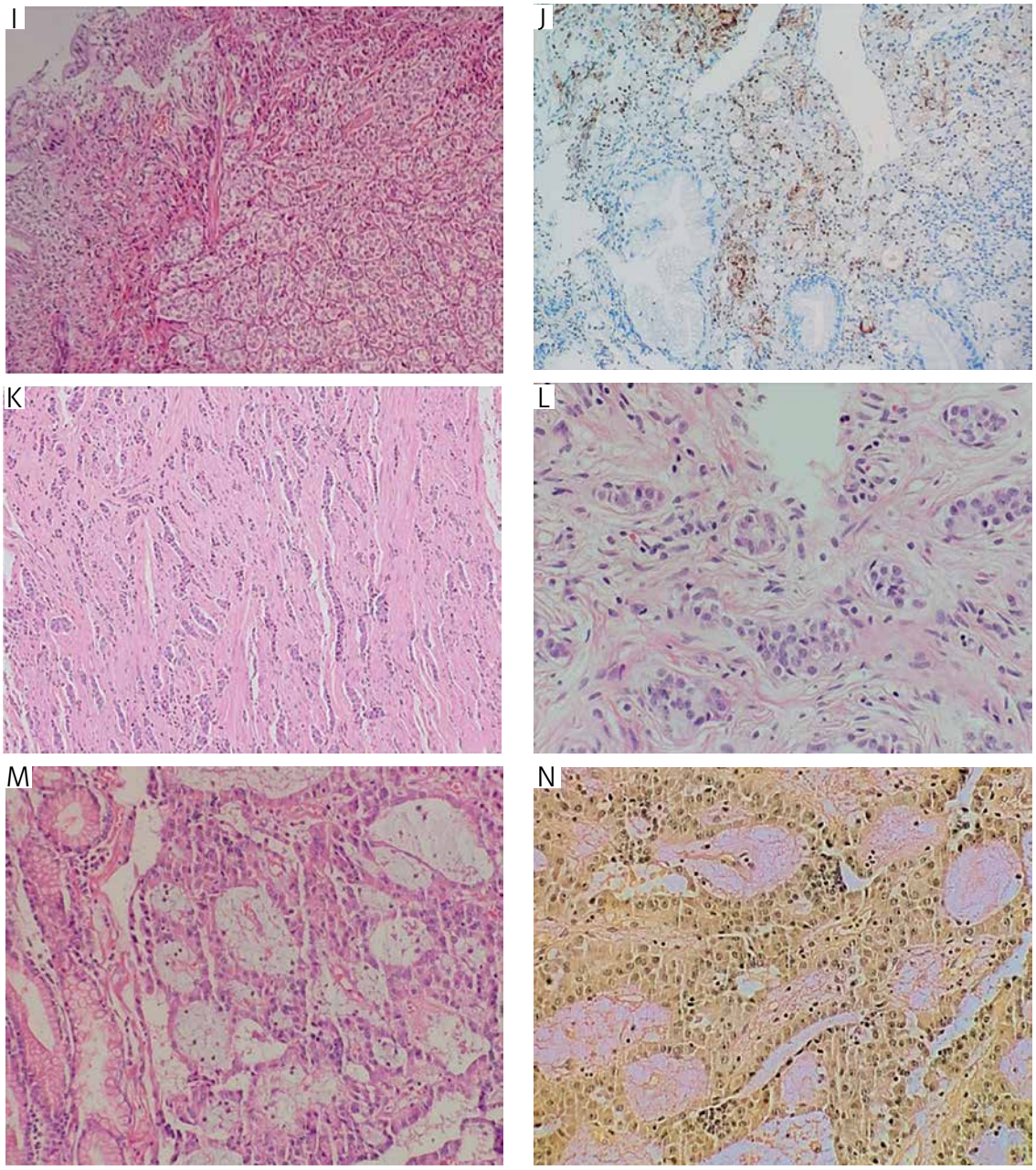

Fig. 3. Cont. I, J) Paraganglioma-like variant NET (i, HE 40×). Positive with S100 immunohistochemical stain (j, S100 100×). K) Mammary tubulo-lobular carcinoma-like variant: tumor cells form small cords or small tubular structures within the sclerotic stroma (HE 40×). L) Tubular variant NET (HE 100×). M, N) Pseudoglandular variant NET (M – HE 100×). Gland-like structures containing mucinous material in their lumens but no demonstrable intracytoplasmic mucin (N – mucicarmine 100×)

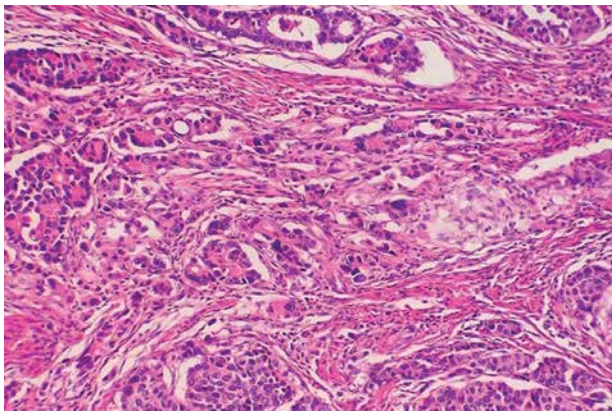

Fig. 4. In the center of the image, there is a limited number of cells with pleomorphic nuclei (HE 200×)

sion 26.0. Armonk, NY: IBM Corp.) was used for all statistical analyses. A *p*-value of less than 0.05 was accepted as statistically significant.

Results

When the demographic information of the patients was analyzed, 133 (52%) patients were determined to be female (F/M ratio: 1.08), and the mean age was 47 ±20 years (range 5–87). The mean Ki-67 proliferation index was 8 ±19 (0–99) and the mean mitotic count was 3 ±8 (0–50). The mean age increased proportionally with increasing grade (Table I). Two hundred and forty-three (94.9%) patients had NETs and 13 (5.1%) patients had NECs. The grade

Table I. Clinicopathologic features of gastrointestinal tract neuroendocrine neoplasms

Parameters	NET Grade 1	NET Grade 2	NET Grade 3	NEC	Total
Gender, *n* (%)					
Female	103 (53.9)	24 (57.1)	3 (30)	3 (23.1)	133 (52)
Male	88 (46.1)	18 (42.9)	7 (70)	10 (76.9)	123 (48)
Age	44.4	48.6	68.9	66.4	47.1
Age (range)	(5–87)	(6–87)	(53–81)	(51–58)	(5–87)
Age (mean)	44 ±20	49 ±20	69 ±9	66 ±10	47 ±20
Biopsy method, *n* (%)					
Endoscopic	96 (50.3)	18 (42.9)	2 (20)	10 (76.9)	126 (49.2)
EMR	5 (2.6)	3 (7.1)	–	–	8 (3.1)
Resection	90 (47.1)	21 (50)	8 (80)	3 (23.1)	122 (47.7)
Organ, n (%)					
Esophagus	–	–	–	2 (15.4)	2 (0.8)
Stomach	88 (46.1)	20 (47.6)	2 (20)	8 (61.5)	118 (46.1)
Small bowel	13 (6.8)	6 (14.3)	6 (60)	1 (7.7)	26 (10.2)
Large bowel	9 (4.7)	3 (7.1)	2 (20)	2 (15.4)	16 (6.2)
Appendix	81 (42.4)	13 (31.0)	–	–	94 (36.7)
Ki-67 index	1.24 (0–2) 1 ±0	6.19 (3–18) 6 ±4	51.5 (25–80) 52 ±20	73.80 (40–99) 74 ±21	7.69 (0–99) 8 ±19
Mitotic count	0.69 (0–1) 1 ±1	2.83 (0–9) 3 ±2	21.8 (4–40) 22 ±13	30.54 (20–50) 31 ±10	3.38 (0–50) 3 ±8
pT Stage, *n* (%)					
pT1	144 (75.4)	25 (59.5)	3 (30)	10 (76.9)	182(71.1)
pT2	14 (7.3)	–	–	–	14 (5.5)
pT3	31 (16.2)	13 (31)	6 (60)	2 (15.4%)	52 (20.3)
pT4	2 (1.1)	4 (%9.5)	1 (10)	1 (7.7)	8 (3.1)
Metastasis, *n* (%)					
Yes	3 (1.6)	6 (14.3)	3 (30)	3 (23.1)	15 (5.9)
No	188 (98.4)	36 (85.7)	7 (70)	10 (76.9)	241 (94.1)
Overall cohort morphology, *n* (%)					
Classic	126 (66)	22 (52.4)	6 (60)	–	154 (60.2)
Oncocytic	5 (2.6)	2 (4.8)	–	–	7 (2.7)
Hepatoid	3 (1.6)	–	1 (10)	–	4 (1.6)
Lipid-rich	1 (0.5)	1 (2.4)	–	–	2 (0.8)
Discohesive- plasmacytoid	9 (4.7)	6 (14.2)	1 (10)	–	16 (6.3)
Pseudoglandular/tubular	39 (20.4)	9 (21.4)	2 (20)	–	50 (19.5)
MTLC-like	4 (2.1)	1 (2.4)	–	–	5 (1.9)
Paraganglioma-like	4 (2.1)	1 (2.4)	–	–	5 (1.9)
Small cell NEC	–	–	–	10 (76.9)	10 (3.9)
Large cell NEC	–	–	–	3 (23.1)	3 (1.2)
Total	191 (74.6%)	42 (16.4%)	10 (3.9%)	13 (5.1%)	256 (100%)

G – grade, MTLC-like – mammary tubulo-lobular carcinoma-like, NEC – neuroendocrine carcinoma

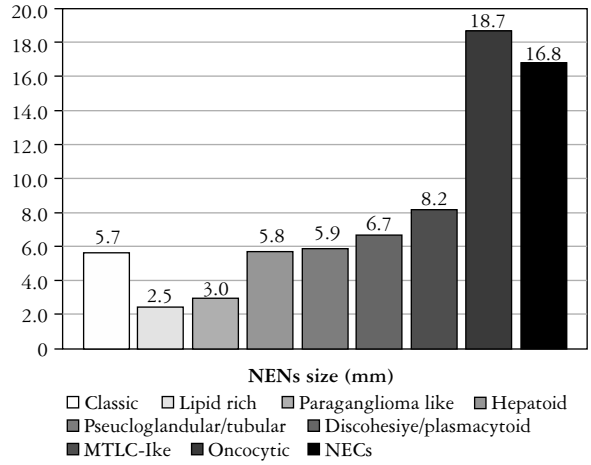

Fig. 5. Median sizes of morphologic variants, classic neuroendocrine tumors and neuroendocrine carcinoma

distribution of NETs was as follows: Grade 1: 78.6% (191 cases), Grade 2: 17.3% (42 cases), and Grade 3: 4.1% (10 cases). While only NECs were found in the esophagus, only Grade 1 and Grade 2 NETs were found in the appendix. When the organ distribution of the NENs was analyzed, the highest rate of NECs was found in the esophagus (100% of esophageal NENs), followed by the large bowel (2 cases, 12.5% of large bowel NENs). Grade 3 NETs were most commonly found in the small bowel (6 cases, 60% of Grade 3 NETs, 23.1% of small bowel NENs). The mean Ki-67 proliferation index was 52 ±20 in Grade 3 NETs and 74 ±21 in NEC cases. The liver was the most common metastatic site ($n = 5$). Liver metastases were Grade 1 in 2 cases, Grade 2 in 1 case, and NEC in 2 cases. Apart from the liver, lymph node metastasis was found in 4 cases (Grade 1 in 1 case, Grade 3 in 2 cases, and NEC in 1 case), peritoneal metastasis in 3 (Grade 2 in 2 cases and Grade 3 in 1 case), small bowel metastasis in 1 (Grade 2), appendix metastasis in 1 (Grade 2), and abdominal wall metastasis in 1 (Grade 2). The clinicopathological data of all cases are given in Table I.

The median tumor size was 6.7 mm (0.3–80 mm) in all NENs. The median sizes of morphological variants, classical NETs, and NECs are shown in Figure 5. The median size of NETs in resection materials was 9.7 mm (1–60 mm). Different morphological variants were detected in 89 (36.6%) cases.

Regarding the organ distribution of the variants differing from the classical NET morphology, the most common organ with morphological variants was the stomach (49.9% of the stomach NETs), and the least common organ was the small bowel (16% of the small bowel NETs). The distribution of NETs with morphological variants according to the organs is shown in Table II.

Discohesive/plasmacytoid, lipid-rich, oncocytic, and hepatoid variants, which are expected to exhibit aggressive behavior, were more frequent in Grade 2 (28.6–50%) and Grade 3 (6.3–25%). The oncocytic variant was the only variant with a pT4 stage, and the risk of metastasis was much higher (42.9%) compared to that of the other variants (Table III).

Less aggressive group: Five cases (5.6%) of paraganglioma-like variants were included in this category. In this group, the median age was 38.8 years (F/M: 1.5), the median tumor size was 3 mm, the median Ki-67 proliferation index was 2.2, and the median mitotic count was 0.8.

Indeterminate behavior group: fifty-five cases (61.8%), including the pseudoglandular/tubular and MTLC-like variants, constituted this category. The median age was 39.2 years (F/M: 0.8), the median tumor size was 6.2 mm, the median Ki-67 proliferation index was 4.3, and the median mitotic count was 2.5.

More aggressive group: Twenty-nine cases (32.6%) of oncocytic, hepatoid, lipid-rich, and discohesive/plasmacytoid variants were included in this category. The median age was 51.6 years (F/M: 1.4), the median tumor size was 9.2 mm, the median Ki-67 proliferation index was 6%, and the median mitotic count was 1.9.

The overall median age of patients with morphological variants was 43.2 years. There was a statistically significant relationship between the morpho-

Table II. Distribution of morphological variant neuroendocrine tumors by organ

Morphological variants /organ	Stomach	Small bowel	Large bowel	Appendix	Total
Pseudoglandular/tubular, *n* (%)	22 (48.9)	–	2 (40)	26 (74.3)	50 (56.2)
Discohesive-plasmacytoid, *n* (%)	11 (24.5)	2 (50)	1 (20)	2 (5.7)	16 (18)
Oncocytic, *n* (%)	5 (11.2)	1 (25)	–	1 (2.9)	7 (7.9)
MTLC-like, *n* (%)	1 (2.2)	–	2 (40)	2 (5.7)	5 (5.6)
Paraganglioma-like, *n* (%)	2 (4.4)	1 (25)	–	2 (5.7)	5 (5.6)
Hepatoid, *n* (%)	2 (4.4)		–	2 (5.7)	4 (4.5)
Lipid-rich, *n* (%)	2 (4.4)	–	–	–	2 (2.2)
Total, *n* (%)	45 (100)	4 (100)	5 (100)	35 (100)	89 (100)

MTLC-like – mammary tubulo-lobular carcinoma-like

Table III. Distribution of morphological variants according to grade, stage, and metastasis

Parameters	Discohesive/ plasmacytoid	Lipid-rich	Onco-cytic	Hepa-toid	Paraganglioma-like	Pseudoglandular/ tubular	MTLC-like
NET grade, *n* (%)							
I	9 (56.3)	1 (50)	5 (71.4)	3 (75)	4 (80)	39 (78)	4 (80)
II	6 (37.5)	1 (50)	2 (28.6)	–	1 (20)	9 (18)	1 (20)
III	1 (6.3)	–	–	1 (25)	–	2 (4)	–
pT stage, *n* (%)							
I	14 (87.5)	2 (100)	5 (71.4)	2 (50)	5 (100)	33 (66)	2 (40)
II	–	–	–	1 (25)	–	8 (16)	0
III	2 (12.5)	–	–	1 (25)	–	9 (18)	3 (60)
IV	–	–	2 (28.6)	–	–	–	–
Metastasis, *n* (%)							
Yes	15 (93.8)	2 (100)	4 (57.1)	4 (100)	5 (100)	48 (96)	5(100)
No	1 (6.2)	–	3 (42.9)	–	–	2 (4)	–

MTLC-like – mammary tubulo-lobular carcinoma-like, NETs – neuroendocrine tumors

Table IV. Relationships of morphological variant groups with age, tumor size, organ, stage, grade, and metastasis

Parameters	Less aggressive		Indeterminate behavior		More aggressive		*p*-value
Median age	38.8 (19–80)		39.2(6–73)		51.6(12–87)		0.011
Median size [mm]	3.0 (2–5)		6.2(1–22)		9.2(1–60)		0.210
Organ, *n* (%)							
Esophagus	0	0.0	0	0.0	0	0.0	0.011*
Stomach	2	40.0	23	41.8	20	69.0	
Small bowel	1	20.0	0	0.0	3	10.3	
Large bowel	0	0.0	4	7.3	1	3.4	
Appendix	2	40.0	28	50.9	5	17.2	
pT stage, *n* (%)							
I	5	100.0	35	63.6	23	79.3	0.099*
II	0	0.0	8	14.5	1	3.4	
III	0	0.0	12	21.8	3	10.3	
IV	0	0.0	0	0.0	2	6.9	
NETs grade, *n* (%)							
I	4	80.0	43	78.2	18	62.1	0.591*
II	1	20.0	10	18.2	9	31.0	
III	0	0.0	2	3.6	2	6.9	
Metastasis, *n* (%)							
No	5	100.0	53	96.4	25	86.2	0.174*
Yes	0	0.0	2	3.6	4	13.8	

NETs – neuroendocrine tumors
** Fisher's exact test p-value*

logical groups and age ($p = 0.011$). Accordingly, the median age was 38.8 years in the less aggressive group, 39.2 years in the behaviorally uncertain group, and 51.6 years in the more aggressive group.

The morphological groups and their median sizes are presented in Table IV. Although there was no statistically significant difference in the median size between the less aggressive group and the more ag-

Table V. Distribution of metastatic disease by organ

Parameters	Organ				Total	*p*- value
	Stomach	Small bowel	Large bowel	Appendix		
Metastasis, *n* (%)						
No	105 (95.5)	22 (88)	11 (78.6)	93 (98.9)	231 (95.1)	0.03
Yes	5 (4.5)	3 (12)	3 (21.4)	1 (1.1)	12 (4.9)	
Total	110 (100)	25 (100)	14 (100)	94 (100)	243 (100)	

Table VI. Comparison between more aggressive group and the cohort

Parameters	More aggressive	NET cohort	*p*-value
Median age	51.9	45.4	0.117
Median size [mm]	9.2	5.8	0.025
Median Ki-67 (%)	5.97	3.9	0.343
Median mitosis	1.86	1.93	0.942

gressive group, the more aggressive group was larger in size.

The difference between morphological groups regarding the organ in which they were found was statistically significant ($p = 0.011$). This significant difference was due to the concentration of the more aggressive group in the stomach and small bowel and the indeterminate group in the appendix. The differences between the variants regarding pT stage, grade, and metastasis were not statistically significant ($p > 0.05$). Although no statistically significant difference was observed, metastasis was not seen in the less aggressive group, while it was more frequent in the more aggressive group, as expected (Table IV). When the groups were compared according to Ki-67 and mitosis, the results were not statistically significant ($p = 0.670$ and $p = 0.782$, respectively).

When the appendiceal tubular variant was separated from the pseudoglandular variant and included in the less aggressive group, there was a statistically significant difference between the groups according to organs and pT stage ($p = 0.00$ and $p = 0.010$, respectively). However, there was no significant difference between morphological groups regarding grade and metastasis ($p > 0.05$). When we included the appendiceal tubular variant in the less aggressive group, the Ki-67 and mitotic count averages of the less aggressive group were 1.4 and 0.7, respectively. On the other hand, the corresponding averages of the indeterminate group were 6 and 3.6, respectively. However, the difference was not significant ($p = 0.174$ and $p = 0.178$, respectively).

Metastatic disease occurred most frequently in large bowel NETs (21.4%), followed by small bowel (12%) and stomach (4.5%) NETs. Metastasis was least common in appendix NETs (1.1%). The rates of metastasis development differed significantly according to the organs ($p = 0.03$) (Table V).

When the difference in chromogranin levels by organ was analyzed, the rate of negativity was found to be higher in the colon than in other organs. Chromogranin was negative in 37.5% of colon NENs, 5.3% of appendix NENs, and 3.8% of small bowel NENs. In the stomach and esophagus, positive staining of chromogranin was observed in all cases ($p < 0.01$).

In comparing the more aggressive group and the entire NET cohort, statistically significant differences were seen in tumor size, metastasis, and organ of origin (Table VI).

Grade 2 had a 10.44-fold higher risk for metastasis development than Grade 1; this result was statistically significant ($p = 0.001$). Similarly, Grade 3 increased the risk of metastasis by approximately 22 times compared to Grade 1. Male patients were found to have a 2.26-fold higher risk of metastasis development than female ones. Regarding age, an increase of 1 year of age increased the risk of metastasis by approximately 1.051 times ($p = 0.004$), and for the tumor size variable, an increase of 1 year of age increased the risk of metastasis by approximately 1.095 times ($p < 0.001$). For the Ki-67 variable, an increase of 1% increased the risk of metastasis by approximately 1.027-fold ($p = 0.002$), while for the mitotic count variable, an increase of 1 mitosis/2 mm^2 enhanced the risk of metastasis by approximately 1.074-fold (a statistically significant difference with $p < 0.001$) (Table VII). The number of metastatic patients was not sufficient to establish a multivariate model.

Discussion

In our study, different morphological variants were detected in 36.6% of GI-NETs. In 2 separate studies on Pan-NETs, this rate was 57.1% and 62.5%, respectively, which is higher than that of GI-NETs [8, 9]. Although the frequency of morphological variants is higher in Pan-NETs, our study has shown that they are also present in GI-NETs at a considerable rate. The peliotic/angiomatous variant, sclerosing variant, ductulo-insular variant, and pleomorphic variant, which are rare morphological variants, were not found in GI-NETs. Some of these variants may

be seen in larger series; alternatively, some of them may be pancreas-specific variants. The most common organ with morphological variants in our series was the stomach. In the distribution of morphological variants according to organs, pseudoglandular/tubular variants were most frequently found in the stomach and appendix, discohesive plasmacytoid variants in the small bowel, and pseudoglandular/tubular and MTLC-like variants (with equal frequency) in the large bowel.

The most important feature of morphological variants for pathologists is that they cause diagnostic difficulties. Case reports and small series on different morphological variants of GI-NETs can be found in the literature [12–27]. It is noteworthy that the diagnostic confusion caused by these variants is the main issue presented. For example, since the lipid-rich variant has clear cytoplasm, it has been observed to cause differential diagnosis difficulties with clear cell tumors, primarily renal cell carcinoma, cholesterol polyps in the gallbladder, and goblet cell adenocarcinoma in the appendix [8, 12, 13, 23].

Liver metastases of the variant with hepatoid differentiation morphologically resemble hepatocellular carcinoma and may show the expression of immune markers such as Hep-Par1 and arginase [24]. The most important aspect of the differential diagnosis is the suggestion that the tumor may be a NET with hepatoid morphology, along with a demonstration thereof using neuroendocrine markers. The plasmacytoid variant is one of the morphological variants that may require a differential diagnosis because of its similarity to hematological malignancies [8, 26]. In differentiating the paraganglioma-like variant from paraganglioma, pancytokeratin positivity, together with its localization, is a useful guide [26]. The mammary tubulo-lobular carcinoma-like variant may give the impression of breast carcinoma metastasis or adenocarcinoma infiltration. In this case, both neuroendocrine markers and immune markers such as GATA3 and SOX10, which indicate breast primary, are helpful in differential diagnosis [8].

Misdiagnosis of the pseudoglandular/tubular variant as adenocarcinoma is a well-known pitfall of NETs [27]. Neuroendocrine cells have cytoplasmic granules containing chromogranin A, synaptophysin, neuron-specific enolase, CD56, PGP9.5, and INSM. Chromogranin A and synaptophysin are the most commonly used immunomarkers for diagnostic confirmation in daily practice. However, their expression may vary in NETs of different regions and grades [28]. Especially in NETs originating from the large bowel and appendix, loss of the neuroendocrine phenotype brings about diagnostic difficulties. In our study, a 37.5% loss of chromogranin expression was observed in the large bowel ($p < 0.01$). Although this does not cause problems in NETs with classical morphology, care should be taken regarding morphological variants. The differential diagnosis becomes more difficult for colon NETs, which may show chromogranin negativity and prostate-specific acid phosphatase (PSAP) positivity and have morphological variants different from the classical morphology [27, 28]. The most important issue in differentiation is to know that one may encounter these variants and to use more than one neuroendocrine immunomarker to confirm the diagnosis.

Table VII. Univariate logistic regression analysis results with odds ratio and 95% confidence interval showing the risk factors affecting metastasis

Univariate logistic regression analysis results		
Parameters	**OR (95% CI)**	***p*-value**
Gender (male)	2.265 (0.752–6.826)	0.146
NEC		
Grade 1	5.775 (1.403–23.766)	0.015
Grade 2	10.444(2.497–43.690)	0.001
Grade 3	22.118 (5.075–96.399)	< 0.001
Age	1.051 (1.06–1.087)	0.004
Size	1.095 (1.052–1.140)	< 0.001
Ki-67 index	1.027 (1.010–1.044)	0.002
Mitotic count	1.074 (1.035–1.114)	< 0.001

NEC – neuroendocrine carcinoma, OR – odds ratio

When encountering high-grade NECs in the GI tract, it is necessary to differentiate between well-differentiated NETs and poorly differentiated NECs, which are two different members of the same family. Neuroendocrine tumors and NECs are neoplasms with different clinical behaviors, treatment responses, radiological findings, and genetic characteristics [7]. Neuroendocrine carcinomas in the GI tract have mutations in TP53 and RB1, which are commonly involved in the pathogenesis of adenocarcinoma. *RB1* gene mutations are more common in small-cell GI-NECs than in large-cell GI-NECs. Apart from these, APC mutations similar to adenocarcinomas have also been reported in GI-NECs. None of these findings were found in NETs [29].

The incidence of NECs, which are rare in the GI tract, was 5.1% in our series. Small-cell morphology was approximately 3 times more frequent than large-cell morphology. In our study, the highest frequency of NECs was in the esophagus (all esophageal NECs), followed by the large bowel (12.5% of large bowel NECs), stomach (6.8% of gastric NECs), and small bowel (3.9% of small bowel NECs) [27, 30, 31]. These findings are consistent with the literature. Neuroendocrine carcinomas were not found in the appendix. Neuroendocrine carcinomas constitute more than 90% of NENs, which encompass

0.04–1% of malignancies seen in the esophagus, as in our study [30]. Two cases found in the esophagus in our series had small-cell morphology and were located in the lower one-third of the esophagus, and one of them expressed TTF1 [27, 31]. In our study, 2 NECs found in the colon were localized in the rectum and sigmoid colon, and both of them were large-cell NECs. Their large-cell morphology is consistent with the literature [32].

The lack of easily recognizable morphological criteria makes the distinction between Grade 3 NETs and large-cell NECs difficult. It has been reported that tumors containing lower-grade NET areas and abnormal expression of p53 and RB1 in resection materials may be helpful in this distinction. In addition, the Ki-67 index does not exceed 80% in well-differentiated NETs and 72 ±20% in NECs. However, in some cases, Ki-67 ratios of Grade 3 NETs and NECs overlap [33, 34]. In our series, the mean Ki-67 proliferation index of NECs was 74 ±21 (40–99) and 52 ±20 (25–80) in Grade 3 NETs ($p = 0.018$) [31]. With these findings, a Ki-67 index above 80% supports the diagnosis of NEC.

Lymph node metastasis and liver metastasis are considered the most important prognostic factors in NENs [10]. In our study, the liver was the most common site of metastasis. Metastatic disease was caused most frequently by large bowel NETs (21.4%) and least frequently by appendix NETs (1.1%; $p = 0.03$). In our study, the prognosis was evaluated based on the presence of metastasis. Tumor size, grade, Ki-67, and mitotic count were found to be the most powerful tools to predict prognosis ($p < 0.05$). However, it is noteworthy that all NETs had metastatic capacity despite their low grade and small size. These findings strongly support the need for different prognostic factors for the better classification of NETs [8–10]. In this study, in which we investigated the effects of morphological variants on prognosis in GI-NETs, the oncocytic variant was found to have a higher metastasis capacity independent of grade. In a study by Xue *et al.*, the oncocytic variant showed the most frequent lymph node metastasis and had a higher recurrence risk than other members of the more aggressive group [8]. Another study reported that oncocytic variant Pan-NETs showed clinically aggressive behavior. It was suggested that these should be considered in the differential diagnosis, especially when a metastatic lesion is encountered [35]. We report that the variant with oncocytic morphology originating from the GI tract was the prominent variant having a negative prognostic value.

In our study, the only member of the less aggressive group was the paraganglioma-like variant. Cases in this group were low grade, as expected. The paraganglioma-like variant, which had no metastasis and all cases of which were stage pT1, was the most promising variant in terms of having a positive prognostic value. The less aggressive group had no metastasis, while the more aggressive group had metastatic disease, as expected. However, the difference between the groups was not statistically significant ($p = 0.174$). We attribute this non-significant result to the limited number of cases in the less aggressive group.

In comparing the more aggressive group and the overall cohort, statistically significant results were obtained regarding tumor size, metastasis, and organ location (Table VI). Interestingly, in this comparison, although the median Ki-67 value was higher in the more aggressive group as expected, the average mitotic count was found to be equal to the overall cohort in the more aggressive group with higher metastatic capacity. The mitotic count, which affects tumor grade, is negatively affected by fixation and is found to be lower than the Ki-67 index, which is not affected by pre-analytical factors [9, 11]. We support the conclusion that the Ki-67 index is more effective than the mitotic count in determining the grade of NETs.

Except for the lipid-rich variant, each morphological variant constituting the more aggressive group exhibited more aggressive features than other GI-NETs. They had a larger tumor size and a higher T stage, Ki-67 index, and metastasis capacity. The oncocytic variant, in particular, was found to have more aggressive behavior, in accordance with the literature [8, 35].

Conclusions

This study demonstrates that GI-NETs have considerable morphological diversity. Aggressive variants, with the exception of the lipid-rich type, were observed to generally form larger tumors and had a higher T stage, a higher grade, and more metastases, especially the oncocytic variant. In contrast, the less aggressive variant, the paraganglioma-like variant, was found to have a smaller size, a lower proliferative index, and a more benign clinical course. The presence of tumors with classical morphology and low-to-moderate-grade tumors with a tendency to metastasize is evidence of the continuing uncertainty regarding NETs. We believe that this situation results from the organ in which the NET develops, tumor size and grade, and the multiple models of genetic alterations that are still being investigated. We believe that all these variables should be evaluated together. Furthermore, we conclude that identifying morphological variants is important for the prognosis and management of the disease and that certain variants have a place in the histopathologic evaluation of GI-NETs. Although the importance of morphological findings is not yet widely accepted, more studies on morphological variants with aggressive behavior will shed more light on this subject in the future.

Disclosures

1. This study was approved by the City Training and Research Hospital Clinical Research Ethics Committee (Protocol No: 916/2023).
2. Assistance with the article: None.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

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