

To the Editor

According to your comments:

1. I used a uniform presentation for figures showing the same or similar contents.
Therefore,
 - a. The legend of figure 1 has been corrected as " Figure 1: Examples of histopathological and immunohistochemical findings in gastrointestinal MiNENs. A: Gastric MiNEN composed of a NET (lower left) intermingled with an adenocarcinoma (X400) B: Colonic MiNEN constituted from a neuroendocrine carcinoma and an adenocarcinoma (x200). C. Diffuse immunostaining with synaptophysin in the neuroendocrine component of a colonic MiNEN (x200) D. The adenocarcinoma component of this MiNEN shows diffuse positivity with CK20 (x400)."
 - b. The legend of figure 2 has been corrected as" Figure 2: Histopathological pitfalls in the diagnosis of MiNEN of the pancreas. A: A ductal adenocarcinoma of the pancreas surrounding and invading an islet in the background of chronic pancreatitis (x200). The islet has regular contours despite an invasion B: A neuroendocrine tumor of the pancreas with entrapped two ductulus without atypia. Such areas should be evaluated carefully to avoid a misdiagnosis of MiNEN(x200)."
 - c. The legend of figure 3 has been revised as Figure 3: Acinar carcinoma of the pancreas A: The tumor is composed of cells that demonstrate the presence of monomorphic nuclei, sometimes forming minute lumens. Tumor cells are in a monolayer with basally located nuclei and have a granular eosinophilic cytoplasm.(x400) B: Bcl-10 expression with higher staining in the apical portion of tumor cells(X400)."
2. I provided decomposable Figures and organized them into a single PowerPoint file.
3. I provided standard three-line tables according to your recommendations.

Review 1:

I would like to thank you for your comments.

Review 2:

Thank you very much for your comments. No doubt they will improve the quality of my manuscript.

While making revisions in line with your criticisms, I noticed that many studies were published, especially for the second half of 2021 (unfortunately, my article was ready for publication at that time). I have summarized this new information into relevant parts of the manuscript (detailed below) and added new references (Refs no.11, 12, 16, 21, 31, 39, 66, 72- 76, 82, 83, 89- 93, 99-101, 105-107,111,116)

- 1) It is commented that many grammar and syntax errors are found in the manuscript. Therefore, the English editing of the revised manuscript is performed by AJE and is certified.
- 2) You criticized that the manuscript lacks specific survival data. For this reason, I performed the literature review once again, and new references containing the latest findings of survival data were added in the section of "Organ-specific clinicopathological findings" of the manuscript., and the necessary revisions were made as follows:
 - a) Under the subheading" Esophagus and Gastroesophageal Junction " (page 13, at the on the last paragraph), the statement" More recently, any statistically significant difference in OS between gastroesophageal GEP MiNEN versus colorectal MiNEN was detected^[66].has been added.
 - b) Under the subheading" Stomach" (page 14) between lines 8-12, the latest data is presented as " Similar to these findings, in a recent study including 401 patients, the 5-year disease-free survival was 51.1%, which was significantly better than that of NEC (47,6%) and worse than that of adenocarcinoma (57,8%). Furthermore, in the same series, advanced stages and lymph node metastasis were independent risk factors related to distant recurrence^[76]."
 - c) Under the subheading "Appendix" (page 15), between lines 21-29, the statements" In a more recent study, the prognosis of 315 patients with MiNEN was compared

with that of other histological subtypes in the appendix, including NETs, NECs, goblet cell carcinoma, signet ring cell carcinoma, mucinous adenocarcinoma and nonmucinous adenocarcinoma, based on the surveillance, epidemiology and end results program 18 registries. The overall 5-year survival rate was 57.4%, and the level of invasion was the only independent factor influencing tumor behavior. In addition, multivariate analysis demonstrated that the prognosis of MiNENs was worse than that of NETs, NECs, goblet cell carcinoma, and mucinous adenocarcinoma but better than that of nonmucinous adenocarcinoma and signet ring cell carcinoma^[83]. "have been added.

- d) Under the subheading " Colon and rectum" (on page 17, lines 3-9), between the last line on page 16 and lines 1-6 on page 17, more recent data presented as" This finding emphasizes the complexity of MiNENs and the need for an accurate morphological description of all components. A recent systematic review also demonstrated that in MiNENs of the lower gastrointestinal tract, the site of origin in those with metastatic disease at diagnosis appeared to influence prognosis. The median survival time was 12.3 months for those with primary colonic tumors versus 11.7 months for those with primary anorectal tumors, with hazard ratios of 1.13 versus 0.80, respectively^[82].
- e) Under the subheading "Pancreas" (on page18, lines 3-11), new data presented as" More recently, lymph node metastasis was indicated as an adverse prognostic factor of disease-specific survival in 7 patients with mixed ductal-neuroendocrine carcinomas^[99]. Similar findings were also observed by Zhang et al. ^[92] in a larger number of patients. Although data for surgically resected cases are very limited in the literature, a cohort study reported that the median survival was 15.3 months and all cases died due to disease^[100]. However, in a recent study evaluating 8 cases with a median follow-up of 21 months, the overall survival was 88 months and the 5-year OS was 58%. In addition, the survival of these tumors was better than that of pancreatic ductal adenocarcinomas; thus, further investigation is warranted^[101]."

- f) Under the subheading "Liver" (on page 19, lines 11-12) a new data was inserted in the statement, " More recently, the 1-year cumulative survival rate of patients was reported to be 53%^[107]."
- g) Under the subheading "Gallbladder and biliary tract" (on page 20, lines 14-19), recent data is presented as" A recent systemic review based on 53 studies to predict the clinicopathological features and prognosis of biliary MiNENs, including gallbladder MiNENs, showed a median overall survival time of 21 months. In addition, radical resection and small morphological subtype were independent prognostic factors associated with higher overall survival, and radical resection (R0) and younger age (<65 years) were associated with higher recurrence-free survival time^[116]."
- 3) You commented that the manuscript lacked updated diagnosis, including molecular, histochemistry, and immunology. As you pointed out in these rare tumors, advances have been made with time but updated slowly. However, I performed a literature search again to find the latest molecular and immunohistochemical findings related to this comment. As a result, I revised the manuscript and presented new data from new references as follows:
- a) Under the heading "THE DIAGNOSIS OF MINEN" (page 6, lines 6-8), the statement" The properties and applications of these markers for neuroendocrine neoplasia (NEN) will be briefly mentioned here." has been added. In addition, the word "However" is inserted in the next phrase, which is followed by the presentation of immunohistochemical findings (lines 9-16) as" Although several biomarkers, including neuron-specific enolase (NSE), CD57, protein gene product 9.5 (PGP 9.5), insulinoma-associated protein 1 (INSM1) and somatostatin receptor subtype 2A (SSTR2A), have been described to date, the most widely used and reliable neuroendocrine markers are chromogranin A, synaptophysin, and CD56^[11,12].In the nonneuroendocrine component, adenocarcinomas express carcinoembryonic antigen, CA 19-9, cytokeratins 7, 19, and AE 1/3. The immunohistochemical features of other tumors that make up

this component are presented below according to their localization in different organs of the GIS^[10-12]."

- b) On the same page (last 4 lines), the statement " Recently, it has been argued that a cutoff value is not mandatory for diagnosing MiNEN because the latest molecular information in the modern classification of these neoplasms has made it possible to demonstrate that both components are clonally related^[16]." has been added.
- c) On page 7, (lines 10-12) the statement" Since neuroendocrine markers can be positive in many nonneuroendocrine tumors considering IHC alone may lead to an overdiagnosis of MiNEN^[19,20]." has been changed as" Since neuroendocrine markers can be positive in many nonneuroendocrine tumors, including poorly differentiated adenocarcinomas, performing IHC alone may lead to an overdiagnosis of MiNEN^[19-21]. "according to the findings of a new reference (ref no 21).
- d) Under the subheading" Subtypes of MiNENs" on page 8 (last 6 lines) and 9 (first 2 lines) the statements " In an elegant study comparing the similarities and differences in genetic alterations between gastric amphicrine carcinomas and MiNENs, Sun et al.^[31] observed that the copy number (CN) characteristics of gastric amphicrine carcinomas were different from those of MiNENs based on a hierarchical clustering analysis, thus supporting that amphicrine carcinoma is a separate entity from MiNENs. In addition, a higher CN level of C5 (complement C5) was observed in amphicrine carcinomas than in MiNENs, suggesting that these tumors might benefit more from C5 inhibitors than MiNENs." have been added to emphasize the difference between amphicrine carcinoma and MiNENs on a molecular basis.
- e) Under the heading "PATHOGENESIS OF MiNEN" (page 9, last 6 lines and page 10, first 3 lines), a new molecular data is presented as" In a more recent molecular study in gastric tumors with targeted DNA sequencing, a great majority of mutations were shared by both ADC and NEC components, and

among them, TP53 was the most commonly mutated gene (69.2%)^[39]. A subset of TP53-wild-type tumors had a microsatellite-unstable phenotype or amplifications in various oncogenes, including ERBB2 and NMYC. While differentially altered genes of ADC components were significantly associated with receptor tyrosine kinase signaling pathways, differentially altered genes of NEC components were significantly associated with the NOTCH signaling pathway, thus providing evidence for a possible clonal origin of ADC and NEC components of MiNENs^[39]."

- f) Under the subheading "Stomach" (page 13, last 6 lines; page 14, first 6 lines), molecular data is presented as " Ishida et al.^[72] compared the molecular pathology of poorly differentiated NEC and MiNEN of the stomach by whole-exome sequencing. The analysis revealed recurrent mutations in 62% of TP53 cases, and they were more frequent in MiNENs than in NECs. Frameshift mutations of APC were observed in two MiNEN cases. In cases of MiNEN, two histological components shared mutations in TP53, APC, and ZNF521, whereas alterations in CTNNB1, KMT2C, PTEN, and SPEN were observed in neuroendocrine components only. They concluded that TP53 is a single, frequently mutated gene in gastric NEC and MiNEN, and alterations in other genes are less common, thus resembling the mutation profiles of gastric adenocarcinomas. Another interesting previous finding is the presence of ATRX gene mutations (primary partial loss) in 37% of cases involving a substantial proportion of gastric MiNEN^[73]. However, these findings should be investigated in further studies."
- g) Under the subheading "Colon and Rectum" (page 16, lines 14-21), new molecular findings described as " Parallel to these findings, a recent case showed that in addition to microsatellite instability due to MLH1 promoter methylation, the same mutations affecting the ARID1A, ASXL1, BLM, and RNF43 genes occur in both components, as determined by a multigene next-generation sequencing panel. On the other hand, BRCA2 has been explicitly

altered in the neuroendocrine area. Although the latter observation suggested that BRCA2 could be a potential new target for MiNEN, the lack of this alteration in the nonneuroendocrine part of the tumor requires further consideration concerning intratumor heterogeneity^[90]."

4. You criticized that update is insufficient. Accordingly, although few, I described the latest findings from my new literature search about MiNENs.
 - a) Under the subheading "Appendix" (page 15, lines 8-14), I inserted new data as " On the other hand, a systematic review showed that among the lower gastrointestinal tract organs, these tumors were most frequently (60,3%) localized in the appendix^[82]. An interesting finding is that the age-adjusted incidence (AAI) for MiNENs increased from 0.01/100,000 person-years to 0.07/100,000 person-years (range 2004-2016), with an annual percentage change (APC) of 13.8%^[83]. This finding can be attributed to the increase in clinical recognition and better diagnostic technologies over the years."
 - b) On the same page (lines 16-18), new demographic data is presented as ". Although recent studies indicate that these tumors do not show sex predilection, new findings that APC shows significant differences according to sex (13.81% in females vs. 12.24% for males) need to be clarified^[83]."
 - c) Under the subheading "Liver" (page 19, line 8), the age ranges have been corrected as " (43-84 years)".
 - d) Under the subheading" Gallbladder And Biliary Tract" (page 19, lines 15-16), the statement" MiNENs of the gallbladder and biliary tract account for 10% of all biliary carcinomas and 2% of all hepatobiliary carcinomas "has been added.
 - e) On the same page (lines 24-27-18), new data presented as " Recent findings indicate that the NEC component of the tumor is composed of large cell NECs in a great majority of cases (59%), and NECs are incidentally discovered during imaging studies without any specific clinical findings^[112].