Romanian Biotechnological Letters *Vol. , No. x,*

ORIGINAL PAPER

Mixed adenoneuroendocrine carcinoma of the gastrointestinal tract- features, diagnosis, management and prognostics

Received for publication, March, 10, 2018
Accepted, June, 6, 2018

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Abstract

Immunohistochemical studies of gastrointestinal tumours have identified a broad spectrum of combinations between exocrine and neuroendocrine components. The wide range of this type of neoplasia varies from adenomas or adenocarcinomas with scattering neuroendocrine cells to classic neuroendocrine tumours with focally disposed of exocrine elements.
Both the exocrine and endocrine elements may have different morphopathological features. The exocrine segment may be an adenoma or even adenocarcinoma with various degrees of differentiation, and the neuroendocrine fraction can be poorly or well differentiated.
The mixed adenoneuroendocrine carcinomas group (MANECs) include those carcinomas in which both neuroendocrine and exocrine components represent at least 30% of the entire tumoral mass. The number of cases mentioned in the literature is small due to the low incidence of this type of pathology, but it can affect any segment of the gastrointestinal tract and depend on the degree of malignancy of each component and its localisation, patients were classified in different prognostic categories.
The current review presents a synthesis of morphopathological, immunohistochemical, molecular and genetic characteristics, clinical-imaging diagnostic elements, management and prognosis specific to this type of cancer.

Keywords: MANEC, gastrointestinal, histological, immunohistochemical, prognosis

Introduction

Gastrointestinal exo-neuroendocrine tumours were first described in 1924 by Cordier (LA ROSA & al.[1];R.CORDIER[2]). The cases reported at that time had very different descriptions such as composite carcinomas, mucin-producing carcinoid, argentaffin cell adenocarcinomas, goblet cell carcinomas, undifferentiated small cell carcinomas, and the list can continue. Thus, in 1987,

Lewin classifies this type of mixed neoplasm in 3 broad categories: collision, combined and amphicrine tumours.
In 2010, the World Health Organization (WHO) established the term "mixed adenoneuroendocrine carcinoma" (MANECs) (G.GRINDI & al.[3]) for neoplasia that exhibits both components, exocrine and neuroendocrine, at least 30% each (LA ROSA & al.[1]; K. CHING-MING[4];R.CARMELO & al.[5]).
Immunohistochemical tests have identified a large number of combinations of the exocrine and neuroendocrine components, ranging from adenomas or adenocarcinomas containing several neuroendocrine cells spread to classical neuroendocrine tumours with focally disposed of exocrine elements (LA ROSA & al.[1]; K. CHING-MING[4]).
However, MANECs are a type of neoplasia rarely encountered in medical-surgical practice, although mixed gastrointestinal tract tumours are found in practice. They are considered carcinomas because both epithelial and neuroendocrine components are malignant tumours. The distal oesophagus and the anal canal were the most frequent sites identified to have a squamous cell carcinoma as an exocrine component (LA ROSA & al.[1]).
Depending on the spatial pattern of its elements, MANECs can be classified into two categories: composite or collision neoplasm. Amphicrine tumours will be distinguished by a divergent immunophenotypic model in which the exocrine and neuroendocrine traits are expressed in the same cell.
All gastrointestinal segments may be affected, but there have also been reported cases of MANEC tumours in the pancreas, bladder and cervix uteri. MANECs are a challenge for the surgeon because in most cases only one of the tumour components is identified, and in incomplete diagnostic circumstances, therapeutic management is insufficient or incorrect.

Materials and Methods

The paper is a review of the specialised literature, made using MDPI Open Access Journals, EPOS, NCBI and other international databases.
In particular, papers from 2005 to 2017 were studied, representing both original articles and literature syntheses.

Results

Symptomatology of MANECs is nonspecific, silent, and often tumours are identified only after exploratory laparotomy for other pathologies or as incidentalomas. A tumour occurs in 5^{th} or 6^{th} decade, and patients are more likely to have metastatic liver pathology. This is why they come for a specific medical assessment.
CT is the first-intention method in identifying and staging MANEC (R.CARMELO & al.[5]). Its sensitivity is proportional to the size of a tumour. Liver metastases were the most commonly recognized elements, and their dimensions may well exceed the size of a primary tumour. Bone radiology and CT identify osteosclerotic or mixed osteolytic-osteosclerotic bone metastases (R.CARMELO & al.[5]; E.K.PAULSON [6]). A primary tumour is small in size; it is difficult to detect by CT and does not show a specific pattern. CT evaluation readily identifies submucosal lesions and liver metastases which enhance the arterial phase due to their high blood supply (F.GIBRIL & al [7]).

A tumour may expand to the mesentery as a tumoral mass containing calcifications or as a stellate/ spiculated mass. The mesenteric vessels may also be affected by the desmoplastic reaction. Tumours can be classified separately depending on how each component develops (Table 1).

Table 1. MANECs- Spatial patterns

Type	Description
Composite	The two types of components develop in separate areas of the tumor
Collision	The exocrine and neuroendocrine parts are mixed and in touch with each other

At barium examination, the appearance of mixed ulcerative and ulcers-infiltrative adenoneurocrine carcinomas is similar to advanced stage gastric cancers. Most of the analysed cases presented regional lymphadenopathy (R.CARMELO & al.[5]).
Desmoplastic reaction at the intestinal level causes intestinal obstruction, vascular thrombosis, infarction, and ischemia of the affected intestinal segment.
Even immunohistochemically adenocarcinomas show among their cells some neuroendocrine elements or if neuroendocrine cancer presents focal non-neuroendocrine components, neither are considered to be MANECs (Table 2).

Table 2. Neuroendocrine markers for each location[6]

	Non-endocrine markers	Location
Chromogranin A	Keratin 7	Gastric
Synaptophysin	Keratin 20	Colorectal
CD56	CDX2	
NSE*	CEA**	

*NSE-Neuron-specific enolase, **CEA- Carcinoembryonic antigen

A particular situation is the goblet cell carcinoma of the appendix, classified by WHO in 2010 both in adenocarcinoma and neuroendocrine tumours, but cannot be classified as MANECs because of the <30% of the neuroendocrine component (LA ROSA & al.[1];N.J.CARR& al [8]).
Considering the malignant nature of its elements, MANEC is regarded as a malignant pathology, and depending on the degree of differentiation of each component, several prognostic categories have been established. However, there is a rare combination of adenomas with a well-differentiated neuroendocrine tumour, known as MANET, whose biological aggression is low. Although the cells of this type of neoplasia have a moderate degree of nuclear atypia and a reduced number of mitotic cell divisions, MANET can metastasise (LA ROSA & al.[1]).
Depending on the potential for malignancy, MANECs are classified into three major categories, listed in Table 3.

Table 3. Classification of gastrointestinal exocrine-neuroendocrine neoplasms according to their degree of malignancy (LA ROSA & al.[1].

Neoplasia Type / Degree Of malignancy	High degree of malignancy	Intermediate degree of malignancy	Reduced degree of malignancy
MANEC*	Adenoma / mixed adenocarcinoma-NEC***	Mixed-Adenocarcinoma G1 / G2 NET Amphicrine carcinoma	
MANET**			Adenoma-NET****

MANEC: mixed adenoneuroendocrine carcinoma; MANET: mixed adenoneuroendocrine tumour; NEC: poorly differentiated neuroendocrine carcinoma; NET: a neuroendocrine tumour, G1 / G2: corresponding to 2010 WHO classification

High-grade malignant MANEC (NEC with small or large cells) is more common in the distal half of the oesophagus, has equal chances of developing in both vertical and horizontal segments of the stomach and it can also occur in the ascending and descending colon (LA ROSA & al.[1];LA ROSA & al [9]; M.J.GAFFEY& al [10]).

This particular type of neoplasia, either combined or composite, has a villous or tubule-villous adenoma/adenocarcinoma, or squamous cell carcinoma, along with a small, intermediate or large cell neuroendocrine carcinoma. Macroscopically, regardless of the location of a tumour, it has a polyploid or an ulcerative stenosis-like appearance, with a diameter varying between 0.5 and 14 cm. It often presents areas of diffuse necrosis. Large or small neuroendocrine cells were positive for synaptophysin and chromogranin A, and the Ki67 index was usually increased (60-90%) (T.TANABE & al. [11]). They were also positive for peptide hormones (somatostatin, ACTH adrenocorticotropic hormone, VIP vasoactive peptide) and nuclear accumulation of p53 antigen. P53 tumour cell antigen was identified in 63% of the analysed cases of gastric cancer and 100% of the ampullary and colorectal tumours (LA ROSA & al.[1];(LA ROSA & al.[12]). The neuroendocrine large cell colorectal subtype showed positive nuclear immunoreactivity for CDX2(LA ROSA & al.[1]).

MANEC CD117 positive colorectal cancers with vascular invasion have been associated with low survival rates (LA ROSA & al.[1];(LA ROSA & al.[12]). Intracellular neurosecretory granules are similar to intrauterine immature proto endocrine cells (C.CAPELA& al.[13]).

The prognosis of patients depends on the type of a tumour and its staging. People with localised disease had better prognosis regarding survival compared to those with metastases. In this way, gastrointestinal MANECs have a better overall prognosis than pure NEC.

Mixed adenocarcinoma-neuroendocrine tumours (MANET) and amphicrine carcinomas are intermediate-grade MANEC (moderately differentiated cells). MANET is neoplasia made up of well-differentiated neuroendocrine cells. Unlike high-grade MANECs, in this type of cancer, the exocrine component is more aggressive than the neuroendocrine part. MANET is a composite tumour consisting of tubular, papillary or mucinous adenocarcinoma areas and NET G1 / G2 areas. The locations where the pathology has been described are the oesophagus, stomach,

Ampulla of Vater, ileum, and colon. 60 cases have been reported in the all literature, with a majority of males aged 65 (LA ROSA & al.[1]). Gastric tumours have the same incidence in the body and gastric antrum. The typical macroscopic appearance is a polypoid mass, between 1,5-10,5 cm in diameter (Y.FUJIYOSHI & al. [14]). The colon can be affected at any level, from the cecum to the rectum. The annular tumours are large (5-7 cm in diameter), and they can cause constriction of the intestinal lumen. The cells of this tumour are moderately differentiated in tubular, papillary or mucous. The well-differentiated neuroendocrine cells are organised in nests, trabeculae or layers. The differences between intestinal and gastric exocrine components are mucin production and the presence of exocrine tumour markers (CEA, epithelial membrane antigen and specific mucins) . (H.R.JR. BATES & al.[15]).
5 cases of gastric MANEC were reported to be associated with chronic autoimmune gastric atrophy and microcarcinoma tumours with enterochromaffin-like intramucosal cells (LA ROSA & al.[1]; M.L.CARUSO & al.[16]; G.PASQUINELLI & al.[17]).
Moreover, MANECs have been identified in distal ileum and cecum in patients with long history of the intestinal inflammatory disease (F.AUBER & al.[18]) and also in patients with Barrett oesophagus (N.R.CARY & al.[9]). Tahara et al. report an average age of 53 years for gastric tumours occurring in patients with chronic gastric atrophy, affecting the entire stomach, following the pattern of lines plastic (Brinton's Disease)(E.TAHARA & al.[20]). Histologically, the tumour contains CEA positive signet ring cells, diffusely arranged and neuroendocrine cells which were positive for chromogranin A and synaptophysin. Isolated forms had better outcomes regarding survival than patients with lymph node metastases who died on average ten months post-gastrectomy)(E.TAHARA & al.[20]).The mean survival rate at five years was 36% (L.H.TANG& al.[21]).
Appendix cancers have been reported more frequently in male patients with a mean age of 50.5 years as diffuse infiltrative solid masses that invade the adjacent caecum and peritoneum.(T.N.MOYANA& al.[22])

A particular type of MANEC-NET appearing in the stomach and the ampulla of Vater is the pancreatic glandular-endocrine composite tumour (acinar cells) where neuroendocrine cells are disposed in substantial polygonal nests surrounded by vessels. Another type of histological pattern described is that with well-differentiated cells that form glands or ducts, surrounded by foveolar gastric cells, positive for cytokeratin 7, CEA or MUC2 (LA ROSA & al.[1]).
The most common sites of MANEC-NET, unfortunately, diagnosed in advanced stages with parietal invasion and ganglion metastases, were those of the intestinal tract. Lymph node. And hepatic metastases show a neuroendocrine component identical to that in a primary tumour.
The adenocarcinomatous component can be represented by many cell types: scirrhous argyrophil cells, signet ring cells, goblet cells.
Amphicrine cells were first described by Feyrter, and subsequently by Ratzenhofer in the gastric mucosa of the rabbit as cells with argentaffin subnuclear granules and mucin vacuoles at the apical pole of the cell. They are sporadic carcinomas (LA ROSA & al.[1]).
Tumours formed by an adenoma and a neuroendocrine component, also known as glandular-carcinoid tumours, have been reported in the terminal ileum, colon, and rectum. Macroscopically, the tumour has a polyploid appearance, with a size from 1.5 to 3 cm. The adenomatous part is made of villous or tubular glands, with a low or high degree of dysplasia. In

the case of composite tumours, the neuroendocrine component is described centrally in the polyp, small sized, nest-disposed, while the adenomatous part is peripherally located. Neuroendocrine cells are argyrophil and positive for chromogranin A, synaptophysin, and in some cases also for somatostatin, serotonin, and glucagon. A tumour invades intestinal mucosa and submucosa, which allows the endoscopic resection of the polyp or a transanal excision surgery. The prognosis of this type of cancer is excellent, with no recurrences recorded (F.MCKEOWN [23]).

McKeown described in 1952 for the first time small cell oesophageal carcinoma derived from APUD cells (F.MCKEOWN [23]). . Immunohistochemically, the cells were positive for chromogranin A, synaptopodin, CD 56 and NSE (Y.ZHU & al.[24];J.P.YUN & al.[25]).

Oesophagus MANECs are rare cancers, extremely aggressive, with poor prognosis regarding survival. There are two main categories: squamous cell carcinoma and adenocarcinoma. There is no standard therapeutic management, and the treatment includes chemotherapy, radiotherapy, and surgical resection J.P.YUN & al.[25]).

Patients with upper gastric tumours present epigastric pain, dysphagia, belching after meals, in addition to signs of neoplastic impregnation (rapid involuntary weight loss, loss of appetite, asthenia, and fatigue). The most significant study of such cases, identified in our literature, relates to the evaluation of 100 cases of gastric cancer (L.NIE[26]).

.A survey made by the Department of Pathology of Nanjing University Hospital, China, tracked 14 patients with MANEC in the upper stomach (L.NIE[26]) . All cases required gastrectomy and lymph node dissection. One patient required partial hepatectomy. In 11 of the cases, the muscular propria was invaded, 9 of the patients had lymph node metastases, and one patient presented liver dissemination. In all cases, the curative resection (R0) was obtained. Four of the tracked patients died in the next 12 months. The study aimed to correlate the histological and immunohistochemical characteristics of a tumour with the prognosis of the patient. Histologically, all 14 cases contained a poorly differentiated neuroendocrine component with a solid, trabecular, tubular or scirrhous aspect, and at least focal necrosis in all cases. The neuroendocrine cells were round- oval, with eosinophilic granules, vesicular or granular nucleus, with or without visible nucleoli. The most sensitive immunohistochemical marker for neuroendocrine cells was synaptophysin (100% of the NEC components were positive), followed by chromogranin A (64%) and CD56 (57%) (L.NIE[26]). Depending on the degree of differentiation of the neuroendocrine component, the cases could be grouped into MANEC-AC / NEC and AC / NET. The first group, in turn, comprises three subgroups: well -differentiated adenocarcinoma (DA), mucin secretory carcinoma (MPC) and poorly differentiated adenocarcinoma (PDA).

A particular subtype of cancer with poor prognosis was hepatoid-NEC adenocarcinoma, found in one case.

The rate of invasion of the muscular propria was higher for AC / NEC than for AC / NET, but lower than for pure NE carcinomas. No significant difference in survival was found, comparing AC / NEC, AC / NET, and NEC. Tumorous grade and the proportion of the neuroendocrine component in the mass have influenced the prognosis negatively. Patients with PDA / NEC had lower survival rates compared to those with MPC / NEC and DA / NEC.

The case of a 30-year-old patient with a family history of colorectal neoplasia, which has been diagnosed with colon MANEC located in her right colon, with lymph node and hepatic metastases, has led physicians in Leuven, Belgium, to study the genetic changes of a tumour. Six somatic modifications of the gene sequences were found. Both components of a tumour were positive for APC gene mutations (Adenomatous polyposis coli-R1096X and L1382fs), KRAS (Kirsten rat oncogene homologous viral sarcoma-G12D), BCL9 (B-cell CLL / lymphoma 9), and FOXP1 P1) (L. VANACKER & al. [27]). The mutation of the SMARCA4 gene (SWI / SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4) was identified only in neuroendocrine cells. Belgian researchers believed that there is a relationship between the two components, and the neuroendocrine phenotype may come from the adenocarcinoma component due to mutations of the SMARCA4 gene. Immediately the following hemicolectomy, the patient received adjuvant treatment with Cisplatin and Etoposide, high doses of Carboplatin, Mitoxantrone, Cyclophosphamide, followed by autologous stem cell transplantation. There were no recurrences.(Y. WENQING & al.[28])
Analyzes of gene mutations were also performed in the case of oesophageal MANEC. TP53 gene mutations, deletions of the RB1 and LOH genes, and PIK3CA, PTEN, KRAS, SOX2, DVL3 and TP63 genes, were present in both tumoral components as well as in the lymph nodes (Y. WENQING & al.[28];B. BALACHANDRA& al.[29]; G. KLÖPPEL& al.[30]).

Discussion

The incidence of MANECs is unknown due to the rarity of this type of a neuroendocrine tumour. A wide variety of combinations of the two components have been reported, from exocrine carcinomas with focal neuroendocrine cells to neuroendocrine neoplasms with scattering exocrine cells. Only those tumours in which each of the two elements exceeds 30% of the tumour volume are admitted as mixed carcinomas. Each part, in turn, may have different morphopathological characteristics, from adenoma to adenocarcinoma with varying degrees of cellular differentiation, and well or poorly differentiated neuroendocrine components. These features significantly influence the evolution of the case.
MANETs are secondary to the simultaneous proliferation of several cell lines or stem cells that differentiate into cells of different categories. In the case of amphicrine tumours, their formation is based on a stem cell capable of divergent differentiation in the same neoplastic cell.
Genetically, gastric and colorectal MANEC formation is closely related to abnormalities of chromosomes 5q, 11q, 17q and 18q (LA ROSA & al.[1]). High-grade NEC malignancies of MANEC have been reported in the oesophagus, stomach, ampulla of Vater, colon and anorectal region (LA ROSA & al.[1]); K. CHING-MING [31]). The average diameter of these carcinomas is 5 cm. The non-neuroendocrine component of the squamous type was found more frequently in oesophageal and anorectal cancers, and adenoma/adenocarcinoma was predominantly found in gastric and colorectal forms (LA ROSA & al.[1]).

Histologically, the poorly differentiated neuroendocrine component resembles small or large cell NEC tumours. The small cell subtype has the cells disposed of in nests or a diffuse manner. The cells have a small amount of cytoplasm, fusiform nuclei with granular chromatin and insensible nucleoli. The mitosis rate is between 20-80 mitotic figures / ten high-power fields (LA ROSA &

al.[1]). High-grade non-small cell tumours are made up of cells with abundant cytoplasm, vesicular nuclei, and prominent nucleoli.

The neuroendocrine component should be immunohistochemically positive for at least 2 of 3 markers used (synaptophysin, chromogranin A, CD56) for making the diagnosis of high-grade MANEC. (LA ROSA & al.[1]); G.GRINDI & al.[3]). The presence of transcription factors (TTF1, ASH1) is not correlated with the patient's prognosis and is not specific for gastrointestinal carcinomas; they are identified in lung, vesicular and urogenital NECs.

No significant differences in survival rate were found between patients with high colorectal grade MANEC and classical NEC patients (LA ROSA & al.[1]); A.PATTA & al. [32])

As the neuroendocrine component is less differentiated and represents a more significant proportion than the exocrine element for a tumour, the evolution and prognosis of the patient are worse. Cases of gastric MANEC in the literature reported a five years survival chance with more prolonged treatment in DA / NEC vs PDA / NEC and MPC / NEC. P53 gene mutation was the most common mutation identified in gastric cancers (Y. WENQING & al. [28]). Some authors claim the origin of a common precursor for the two components of a tumour, but they state their evolution in different cell lines. A similar theory is that of stem cells capable of differentiation in different cell lines. Arguments supporting this assertion are amphicrine cells, expression of at least one exocrine marker in neuroendocrine cells, and expression of neuroendocrine markers in gastric cells (Y. WENQING & al. [28]). Elective treatment for gastric carcinomas is total or partial gastrectomy followed by Roux en Y oesophageal anastomosis. Chemotherapy with Cisplatin and Etoposide are recommended in case of metastases. Gastrectomy is useful at early stages and for obstruction of the oesophagus or pyloric junction. In advanced stages, platinum-based chemotherapeutic agents are indicated as the first line of treatment (A.C.NICOLAE al.[33]).

To predict the evolution of a patient as accurate as possible, the two components of a tumour are analysed and evaluated individually regarding neoplastic grading. Jiang et al. consider that a 20% proportion of the neuroendocrine part amplifies the unfavourable evolution, but Park et al. suggests that already 10% represents a potentially fatal percentage (Y. WENQING & al. [28]). The degree of component differentiation influences the prognosis more than the volume occupied by each component.

Poorly differentiated neuroendocrine colon carcinomas are rare entities, which, if metastasised, have a terrible outcome due to the lack of treatment. The presence of distant metastases contraindicates resection in oncological limits. This type of aggressive carcinoma responds well to chemotherapy with Cisplatin and Etoposide, but the chance of survival remains less than 12 months (B. BALACHANDRA & al. [29]; P.J.STEPHENS [34]). There have been no reports of patients with stage IV MANEC who survived. A retrospective study regarding the efficacy of Cisplatin-Etoposide in the treatment of NEC with liver metastases yielded the following results: 12.5% of cases responded entirely to therapy, and 50% of patients had a partial response (B. BALACHANDRA & al. [29]). The mean survival rate in this study was 4.5 months, and no patient survived for more than 17 months (P.J.STEPHENS [34]) . The Belgian patient referenced in the Results section survived 20 years without relapse due to the association of Cisplatin-Etoposide initial therapy with high-dose chemotherapy regimens with Carboplatin, Mitoxantrone and Cyclophosphamide, followed by autologous stem cell transplantation. The researchers

demonstrated the monoclonal link between exocrine and neuroendocrine components by identifying KRAS mutations in both elements (G.ROBINSON [35]).

The cause of the adenocarcinoma phenotype changing into the neuroendocrine phenotype was the phenomenon of rearranging up to 1000 chromosomal clusters into one or more chromosomes at the same time, known in the Anglo-Saxon literature as "chromothripsis." Practically, in the adenocarcinoma cell DNA, there is a catastrophic mutation that allows the development of aggressive clones of neuroendocrine cells (H.L. WALDUM [36]). The exon sequences of adenocarcinoma and neuroendocrine carcinoma were compared, and the only event produced in their behaviour was the hepatic domain of the SMARCA4 gene (B. BALACHANDRA & al. [29]). The same type of mutation occurs in the case of a medulloblastoma subtype when some Wnt-responsive chromatin remodelling genes are altered (B.G. WILSON & al. [37]). There are several similarities between the neuron and the neuroendocrine cell, which has allowed comparative analysis of their morphology, functionality, and gene profiles (C.POIANA& al. [38] C.POIANA & al. [39]).

It has been established that inactivation of the SMARC4A gene is responsible for the transformation of the exocrine cell into the neuroendocrine carcinoma cell, as in the case of tumours of neuroectodermal origin, including the medulloblastoma .

Other histopathological subtypes can coexist with neuroendocrine carcinoma. The literature describes the association with immature teratoma, for example. Tumours with this type of association carry adverse prognosis. Other factors associated with bad outcome are lack of staining for chromogranin in immunohistochemistry studies. This is usually related to G2 NET. On the other hand, intense staining for somatostatin is associated with adverse outcome. By far, the most important prognostic factor is ki67 proliferation index (D.L. PĂUN & al. [40]).

History of NETs in the same family carries bad prognosis as well.

Conclusion

Gastrointestinal MANEC is a heterogeneous group of tumours with different morphopathological, clinical and prognostic elements depending on the type and degree of differentiation of each component. Diagnostic suspicion is due to secondary symptoms such as obstruction and liver metastases. The most useful imaging method remains CT (MDCT), but evidence and precise diagnosis made on the histopathologic examination.

The central metastasis stations are regional lymph nodes and the liver. The prognosis of mixed adenoneuroendocrine carcinomas is weak, given the malignant nature of both components of a tumour. Both parts of mixed carcinomas can influence the patient's prognosis through their characteristics (size, histology, the degree of differentiation).

Conflict of interest

The authors declare that they have no competing interests. All data can be accessed by e-mailing the correspondence author.

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