# FROM CARCINOIDS TO A BIOLOGICALLY AND PROGNOSTICALLY RELEVANT CLASSIFICATION OF THE NEUROENDOCRINE TUMORS OF THE GASTROINTESTINAL TRACT AND THE PANCREAS

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SUMMARY – Although well established in the medical terminology, the term carcinoid is no longer adequate to cover the entire morphological and biological spectrum of neoplasms of the disseminated neuroendocrine cell system. Instead of "carcinoid" the WHO classification published in 2000 therefore uses the general terms "neuroendocrine tumor" and "neuroendocrine carcinoma." In this review we describe a classification of gastroenteropancreatic neuroendocrine tumors based on the WHO criteria. We also classify and comment on the most important tumor entities. On the basis of localization and of various morphological and biological criteria we distinguish between benign neuroendocrine tumors, tumors with uncertain malignant potential, and tumors showing low grade and high grade malignancy.

Key words: *carcinoids, neuroendocrine tumors, classification, prognosis, diagnosis*

## Introduction

Since 1907, when Oberndorfer differentiated "carcinoid tumors" from the carcinomas of the gastrointestinal tract[1], these tumors have been (and to some extent still are) considered to represent a fairly homogeneous group. Because these tumors are extremely rare it is tempting to lump them together when classifying them, assessing their prognosis and treating them. In the past two decades, however, our knowledge of the development and biological behavior of the gastroenteropancreatic neuroendocrine tumors (GEP-NETs) has increased so greatly, thanks to advances made in clinical and morphological diagnostics, that a more differentiated view of the classification and treatment of the GEP-NETs is required. In this review we will therefore briefly describe the morphological, biological and prognostic peculiarities of the GEP-NETs and present and comment on an adequate classification based on the 2000 WHO classification and other prior reviews on this topic[2-6].

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## Origin and Phenotypical Differentiation of Neuroendocrine Tumors

Phenotypically, the cells of the GEP-NETs belong to the system of disseminated neuroendocrine cells, which Feyerter referred to as "Helle Zellen" (clear cells) and Pears as "APUD cells".[7] These cells are scattered throughout the mucosa of the gastrointestinal tract or form the "islets" in the pancreas described by Langerhans. The term "neuroendocrine" derives from the phenotypical relationship to neural cells in the expression of certain proteins, such as synaptophysin, neuron-specific enolase and chromogranin A. These proteins can be employed as general markers in the clinical and morphological diagnosis of GEP-NETs, because they are to a large extent independent of the cell-specific hormone production. When applying these

markers in immunocytochemical analyses it is important to remember, however, that chromogranin A is not (or scarcely) expressed by certain types of endocrine cells, such as somatostatin cells or very poorly differentiated NET cells, and that neuron-specific enolase frequently leads to unspecific reactions because of the existence of various closely related dimeric isoforms.

Specific markers of the normal and neoplastic NE cells are the hormones that occur in the GEP system. At least 12 different types of endocrine cells are currently known[7]. Strangely enough, although there are so many hormones, for reasons still unknown, only somewhat less than half of the known hormones are expressed in GEP-NETs. Moreover, biologically the organ in which a certain hormone-producing tumor develops appears to make a difference, as illustrated by the example of the duodenal and pancreatic gastrinomas with their differing malignant potential (cf. below).

## Terminology: From "Carcinoid" to Neuroendocrine Tumor

Even today most of the GEP-NETs are referred to as "carcinoids." Oberndorfer coined this term in 1907 to point out that there are epithelial tumors in the gut that have a relatively monotonous structure and are less aggressive in their behavior than carcinomas. In 1963 Williams and Sandler[8] divided the carcinoids according to embryogenetic aspects into foregut (lung, stomach, duodenum, upper jejunum and pancreas), midgut (lower jejunum, ileum, appendix, cecum) and hindgut (colon and rectum) carcinoids. This classification, the first to emphasize clinicopathological differences between the tumor groups composing the GEP-NETs, never was generally accepted in routine diagnostic practice, because it proved too imprecise to be able to distinguish between the different biologically relevant GEP-NET entities[9]. This is particularly evident in case of the neuroendocrine tumors of the foregut, which differ too greatly in their morphology, function and biology to be classified in a single group.

*Table 1. Classification of neuroendocrine tumors of the gastroenteropancreatic system (GEP-NET)*

| | |
|---|---|
| 1a | Well differentiated neuroendocrine tumor |
| 1b | Well differentiated neuroendocrine carcinoma |
| *2* | Poorly differentiated neuroendocrine carcinoma |

In the first WHO classification of endocrine tumors, published in 1980, the term "carcinoid" was applied to most of the neuroendocrine tumors. Only the endocrine tumors of the pancreas and the thyroid, paragangliomas, small-cell lung carcinomas and Merkel cell tumors of the skin were differentiated. The "carcinoids" were divided into enterochromaffin (EC cell), gastrin (G cell) and other unspecified "carcinoids." It proved difficult to apply this WHO terminology, however, because it often led to misunderstandings between pathologists and clinicians. One of the main reasons for these misunderstandings was that the pathologists applied the term "carcinoid" to all tumors with neuroendocrine features, whereas the clinicians understood the term "carcinoid" to mean a serotonin-producing tumor with carcinoid syndrome. Another difficulty arising from the diagnosis of "carcinoid" was related to the heterogeneity of these tumors that was becoming increas-

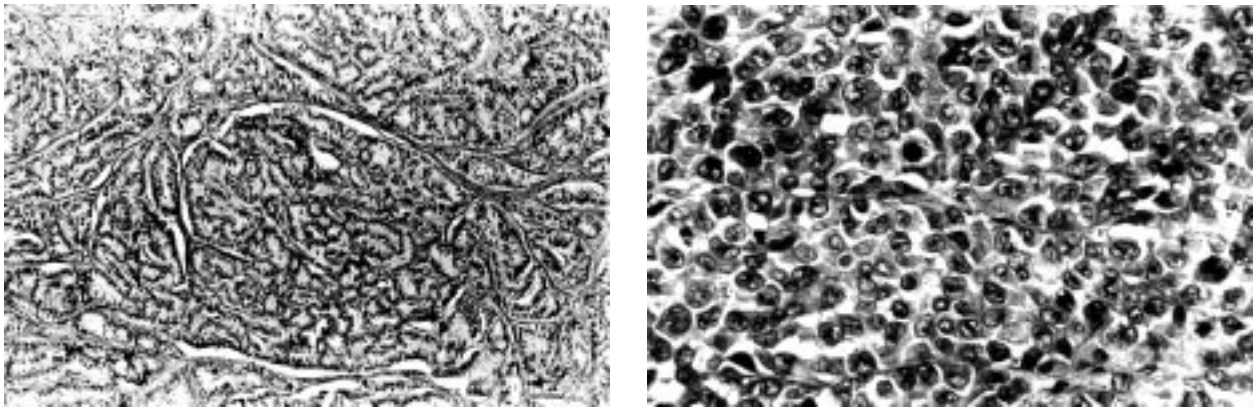

*Fig. 1. a) Well differentiated neuroendocrine carcinoma with a trabecular pattern, b) poorly differentiated neuroendocrine carcinoma*

ingly obvious from more recent publications. It was, e.g., no longer possible to equate a carcinoid of the stomach with a carcinoid of the ileum or rectum or to include among the carcinoids tumors whose atypical histology hardly revealed any features in common with the carcinoids.

For these reasons the neutral and inclusive terms neuroendocrine tumor and neuroendocrine carcinoma were chosen for use in the WHO classification of 2000[10]. A distinction was made between well differentiated neuroendocrine tumors, which show benign behavior or uncertain malignant potential; well differentiated neuroendocrine carcinomas, which are characterized by low grade malignancy, and poorly differentiated (usually small cell) neuroendocrine carcinomas of high grade malignancy (Table 1 and Fig. 1). To prevent misunderstandings, the term "carcinoid" was not (yet) completely abandoned. For the gastroenteric NETs it was used synonymously with the term well differentiated neuroendocrine tumor. The term "malignant carcinoid" was used synonymously with the term well differentiated neuroendocrine carcinoma. In a second step the classification based on this terminology (Table 1) was subdivided on the basis of localization and biology of the tumors, in order to achieve a prognostically relevant classification of the tumors. As far as tumor localization is concerned, the stomach, duodenum (and proximal jejunum), ileum (including the distal jejunum), appendix, colon-rectum and pancreas were distinguished. The morphological/biological criteria employed were tumor size, angioinvasion and proliferative activity, in addition to histological differentiation, the presence of metastases and invasion of adjacent organs. Finally, as a further biological parameter, hormonal activity and association with certain clinical syndromes or diseases were included (Tables 2-6). In the following the GEP-NET entities will be briefly presented and classified according to their prognosis. We will not deal with the very small group of mixed exocrine-neuroendocrine tumors, with the exception of the "goblet cell carcinoids" of the appendix, the best known representative of this rare tumor category. Nor will we go into details of tumor-like lesions, such as ECL cell hyperplasia of the stomach.

## Neuroendocrine Tumors of the Stomach

Four types of NET can be distinguished in the stomach[5, 11]. Type 1 is by far the most frequent type and comprises approx. 70%-80% of all cases[12]. The next most frequent type is type 3 (cf. below), whereas types 2 and 4 are rare. It is likely that type 1 is even more common than previously assumed. The incidence of this gastric NET was not really appreciated prior to the increased application of endoscopy in gastroenterology. It is quite possible that meanwhile type 1 gastric NETs lead the list of the most frequent gastrointestinal NETs. This should at least be borne in mind when quoting the much cited, but outdated, frequency data of Godwin[13] or Modlin and Sandor[14], which emphasize the NETs (carcinoids) of the appendix and the ileum.

Type 1 NETs of the stomach are in most cases small multifocal tumors. During endoscopy multiple small, broad-based, round, polypoid mucosal tumors measuring 0.5-1 cm in diameter are typically found in the corpus of the stomach. Clinically these tumors are not associated with any symptoms, or only with very general gastric symptoms. Mainly women are affected (70%-80% of the cases). Histologically, the tumors are well differentiated, located in the mucosa and submucosa and composed of intensely chromogranin A positive ECL (enterochromaffin-like) cells. These cells, which produce histamine, are specifically recognized by the marker VMAT2 (vesicular mono-amine transporter 2)[15, 16]. A characteristic of type 1 gastric NETs is that they always develop in connection with autoimmune chronic atrophic corpus gastritis (CAG). This is in all probability the result of autoimmune destruction of the specific glands of the corpus mucosa. On the one hand, the loss of parietal cells leads to insufficient production of intrinsic factor and can thus trigger pernicious anemia *via* the decreased resorption of vitamin B12. On the other hand, the loss of hydrochloric acid producing parietal cells causes achlorhydria of the stomach, which for its part stimulates the antral G cells to produce gastrin, causing persistent hypergastrinemia. It is assumed that the hypergastrinemia promotes the growth of the ECL cells of the corpus mucosa so that diffuse to micronodular ECL cell hyperplasia develops, out of which after a latent period of many years the above described multiple ECL tumors arise[6]. No hormonal syndrome develops in the process. The observation that the tumors can occur in only partial atrophic corpus gastritis and the detection of growth factors such as TGF-alpha, bFGF und BCL-2 are indications that hypergastrinemia alone probably does not cause these tumors to develop[6]. The prognosis of these tumors is good, because they are

generally so small that they can be removed endoscopically[17]. Regional lymph node metastases seem to occur only in very rare cases, in which the tumors are larger than 2 cm in size and infiltrate the muscularis propria.

Type 2 gastric NETs occur in association with multiple endocrine neoplasia type 1 (MEN-1), a hereditary, autosomal dominant disorder, in the course of which a Zollinger-Ellison syndrome (ZES) has developed. These gastric NETs occur with approximately equal frequency in men and women. Like type 1 NETs they are usually multifocal and occur in the corpus of the stomach and on the basis of ECL cell hyperplasia. The tumors are generally smaller than 1.5 cm and limited to the mucosa and submucosa. If angioinvasion is found or the tumor is larger than 2 cm and/or has invaded the muscularis propria, it is likely that the tumor has metastasized, as happens in approx. 30% of the cases. The genetic changes associated with MEN-1 (mutations in the MEN-1 gene on chromosome 11q13) in connection with gastrinoma-related hypergastrinemia are probably the basis on which the tumor develops, because so far the development of tumors of the same type in ZES has not been confirmed.

Type 3, or sporadic, NETs of the stomach are not associated with either CAG or MEN-1. They show a predilection for the male sex. These tumors are solitary and do not have any special localization in the stomach. In one third of the cases the tumor is already larger than 2 cm at the time of diagnosis. Histologically, the tumors are predominantly well differentiated and are composed of ECL cells. Tumors with EC (serotonin) cells or gastrin cells, by contrast, are extremely rare[11]. If the tumor is larger than 2 cm, has invaded the muscularis propria and angioinvasion is recognizable it is likely that there will be metastases[17]. In such cases surgical treatment is required.

Type 4 gastric NETs correspond to an undifferentiated solid carcinoma composed of small to medium-sized cells. These tumors are more common in men than in women. There is no relationship to CAG or MEN-1. At the time of diagnosis most of the tumors are already in an advanced stage (tumor diameter more than 4 cm) and show extensive metastases. Immunocytochemically, the tumor cells are positive only for synaptophysin, often with slight focal positivity for chromogranin A. Type 4 NETs of the stomach are treated like normal gastric carcinomas.

Table 2 shows the prognostically relevant classification of gastric NETs with all of the above described entities.

*Table 2. Classification of neuroendocrine tumors of the stomach*

1. Well-differentiated tumor (carcinoid)
   - Benign: nonfunctioning, confined to mucosa-submucosa, nonangioinvasive, ≤ 1 cm in size
     - ECL cell tumor of corpus-fundus (usually multiple) associated with chronic atrophic gastritis (CAG) or MEN-1 syndrome
     - Serotonin-producing or (very rare) gastrin-producing tumor
   - Benign or low grade malignant (uncertain malignant potential): nonfunctioning, confined to mucosa-submucosa, with or without angioinvasion, >1-2 cm in size
     - ECL cell tumor with CAG or MEN-1 syndrome or sporadic
   - Serotonin-producing or (very rare) gastrin-producing tumor
2. Well-differentiated neuroendocrine carcinoma (malignant carcinoid)
   - Low grade malignant: invasion of the muscularis propria and beyond or metastases, >2 cm in size
     - Nonfunctioning: usually sporadic ECL cell carcinoma, rarely in CAG/MEN-1 or serotonin or gastrin-producing
     - Functioning with serotonin-producing carcinoma (atypical carcinoid syndrome) or gastrin-producing carcinoma (gastrinoma)
3. Poorly differentiated neuroendocrine carcinoma
   - High grade malignant

## Neuroendocrine Tumors of the Duodenum and Upper Jejunum

Five types of duodenal neuroendocrine tumors can currently be distinguished. These include duodenal gastrinomas, which make up approx. two thirds of all duodenal NETs, duodenal somatostatinomas, nonfunctioning (i.e. they do not cause a hormonal syndrome) serotonin, gastrin or calcitonin producing tumors, poorly differentiated, predominantly ampullary neuroendocrine carcinomas and duodenal gangliocytic paragangliomas.[2, 3, 6]

Table 3 shows the prognostically relevant classification of the NET of the duodenum with all of the above described entities.

Duodenal gastrinomas are either sporadic or associated with MEN-1 and are combined with a ZES.[18, 19] In both situations these gastrinomas are usually not bigger than 1

*Table 3. Classification of neuroendocrine tumors of the duodenum and upper jejunum*

1. Well-differentiated neuroendocrine tumor (carcinoid)
   - Benign: nonfunctioning, confined to mucosa-submucosa, nonangioinvasive, ≤ 1 cm in size
     – Gastrin-producing tumor (upper part of the duodenum
     – Serotonin-producing tumor
     – Gangliocytic paraganglioma (any size and extension, periampullary)
   - Benign or low grade malignant (uncertain malignant potential): confined to mucosa-submucosa, with or without angioinvasion, or >1 cm in size
     – Functioning gastrin-producing tumor (gastrinoma), sporadic or MEN-1 associated
     – Nonfunctioning somatostatin-producing tumor (ampullary region) with or without neurofibromatosis type 1
     – Nonfunctioning serotonin-producing tumor
2. Well-differentiated neuroendocrine carcinoma (malignant carcinoid)
   - Low grade malignant: invasion of the muscularis propria and beyond or metastases
     – Functioning gastrin-producing carcinoma (gastrinoma), sporadic or MEN-1 associated
     – Nonfunctioning somatostatin-producing carcinoma (ampullary region) with or without neurofibromatosis type 1
     – Nonfunctioning or functioning carcinoma (with carcinoid syndrome)
     – Malignant gangliocytic paraganglioma
3. Poorly differentiated neuroendocrine carcinoma · High grade malignant

cm and are located predominantly in the upper part of the duodenum. Histologically, they show a trabecular/pseudoglandular pattern and immunocytochemically, they are clearly gastrin positive. MEN-1-associated gastrinomas are usually multiple, in contrast to sporadic gastrinomas. In spite of their small size and the fact that they are limited to the duodenal mucosa and submucosa, at the time of diagnosis metastases are often found in the regional lymph nodes. These metastases may be much larger than the primary tumor and may erroneously be considered pancreatic tumors, especially if they are located close to the pancreas[20]. This peculiarity is probably the reason why earlier authors spoke of primary lymph node gastrinomas and why many more pancreatic gastrinomas used to be diagnosed than are today. Whereas metastasis to the regional lymph nodes occurs at an early stage, liver metastases appear to be a relatively late occurrence. In case of pancreatic gastrinomas (cf. the appropriate section), which are practically all sporadic and hence not associated with MEN-1, liver metastases seem to occur earlier than in duodenal gastrinomas[20, 21].

Duodenal somatostatinomas account for approx. 15% of all duodenal NETs. Their preferential localization is in the region of the papilla of Vater or periampullary. If the muscularis propria is invaded, metastasis to the paraduodenal lymph nodes has probably occurred. Histologically, the tumors typically show a glandular pattern with psammoma bodies. Immunocytochemically, somatostatin can be detected in almost all tumor cells. In contrast to pancreatic somatostatinomas, however, no somatostatin syndrome (diabetes, cholelithiasis and diarrhea) develops. Instead, they are often associated with neurofibromatosis type 1. In this situation a bilateral pheochromocytoma can simultaneously develop.

Nonfunctioning duodenal NETs usually consist of serotonin-producing cells. Occasionally there are also tumors with gastrin or calcitonin-positive cells. The prognosis of this group of nonfunctioning tumors is much more favorable than of ZES-associated gastrinomas or ampullary somatostatinomas. Metastases are not to be expected until the tumor extends beyond the submucosa.

Poorly differentiated duodenal carcinomas occur primarily in the region of the papilla of Vater. They are hormonally inactive. At the time of diagnosis advanced metastasis into the regional lymph nodes and the liver has usually occurred. Histologically, the tumors are undifferentiated, often relatively small cell, carcinomas with strong synaptophysin positivity in connection with slight or lacking chromogranin A positivity.

Duodenal gangliocytic paragangliomas occur in the vicinity of the papilla of Vater. Although the tumors are often larger than 2 cm and invade the muscularis propria, they are generally benign. Histologically, they reveal a gangliocytic component in addition to well differentiated neuroendocrine cells, and immunocytochemically, they usually express somatostatin, pancreatic polypeptide and S-100.

## Neuroendocrine Tumors of the Appendix

The NETs of the appendix are, along with ileal NETs, the most frequent GEP-NETs. Their prognosis, however, is much more favorable than that of ileal NETs. They oc-

cur primarily in the tip of the appendix and almost without exception they invade the muscularis propria and, to a greater or lesser degree, the adjoining fatty tissue of the mesoappendix. Nevertheless, regional lymph node metastases are not likely to be found before the tumor reaches a size of approx. 2.5 cm. Histologically and immunocytochemically, the NETs of the appendix are comparable to the ileal NETs with their solid tumor cell formations and their positivity for serotonin and substance P. In addition, very rare enteroglucagon-producing NETs are also found. Because the serotonin-producing appendix NETs practically never metastasize to the liver, the development of a carcinoid syndrome is very rare. The classical appendix NETs must be distinguished from the very rare so-called goblet cell carcinoids, which represent mixed exocrine-endocrine tumors, because the prognosis of the latter is less favorable. Like in the ileum, no poorly differentiated malignant NETs have been described in the appendix so far.

Table 4 shows the prognostically relevant classification of the NETs of the appendix.

*Table 4. Classification of neuroendocrine tumors of the appendix*

1. Well-differentiated neuroendocrine tumor (carcinoid)
   - Benign: nonfunctioning, confined to appendiceal wall, nonangioinvasive, L 2 cm in size
     – Serotonin-producing tumor
     – Enteroglucagon-producing tumor
   - Benign or low grade malignant (uncertain malignant potential): nonfunctioning, invading the mesoappendix, angioinvasive, > 2 cm in size
2. Well-differentiated neuroendocrine carcinoma (malignant carcinoid)
   - Low grade malignant: infiltrating deeply into the mesoappendix, > 2.5 cm in size, or with metastases
     – Nonfunctioning or functioning serotonin-producing carcinoma (with carcinoid syndrome)
3. Mixed exocrine-neuroendocrine carcinoma
   - Low grade malignant
     – Goblet cell carcinoid

## Neuroendocrine Tumors of the Ileum

These frequent NETs develop quite preferentially in the terminal ileum and occasionally in the immediately adjacent cecum, including the ileocecal valve. Histologically, they show a solid ("island-like") pattern. At the time of diagnosis they are commonly larger than 2 cm and have invaded the muscularis propria. This means that they have already metastasized to the regional lymph nodes (Fig. 2).

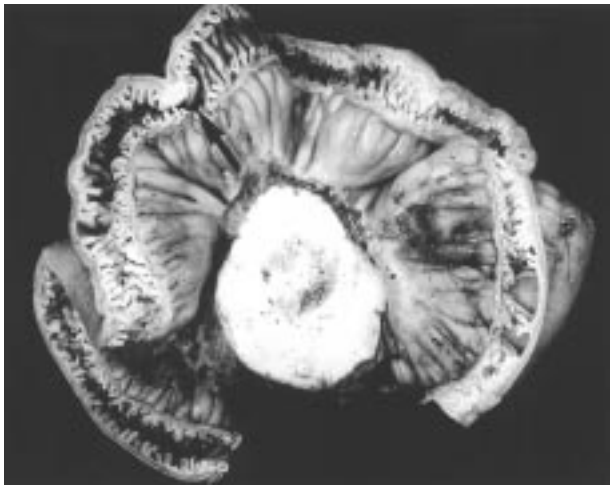

*Fig. 2. Small neuroendocrine carcinoma of the ileum (arrow) with a large regional lymph node metastasis*

In up to 40% of cases the tumors may be multiple. Immunocytochemically, the cells contain serotonin and substance P, kallikrein and catecholamine. Approx. 20% of the patients with ileal NETs have liver metastases in addition to regional lymph node metastases. Only in these patients does a carcinoid syndrome develop. It is characterized by flush, diarrhea and endocardial fibrosis, because serotonin is inactivated by the liver and only enters the circulatory system if there are liver metastases. Poorly differentiated malignant NETs have not yet been described in the ileum.

Table 5 shows the prognostically relevant classification of the ileal NETs.

## Neuroendocrine Tumors of the Colon and Rectum

NETs of the colon are very rare. Histologically, they are poorly differentiated neuroendocrine carcinomas, almost all of which have already metastasized at the time of diagnosis and therefore have a poor prognosis[22]. Immunocytochemically, they are generally synaptophysin positive and may contain single serotonin and somatostatin-positive cells.

Rectal NETs are much more frequent and their prognosis is much better than that of NETs of the colon. They comprise around 10% of all GEP-NETs. Most appear during endoscopy as small (<1 cm), movable submucosal

tumors. Metastases are only likely in tumors that are 2 cm and larger and if they invade the muscularis propria. Immunocytochemically, they are positive for glucagon, glicentin and/or pancreatic polypeptide (PP). In this portion of the gut poorly differentiated neuroendocrine carcinomas are the exception. They have as poor a prognosis as those in the colon and other portions of the gut. Table 5 shows the prognostically relevant classification of the NETs of the colon and rectum.

## Neuroendocrine Tumors of the Pancreas

Most of the endocrine tumors of the pancreas are well differentiated NETs or NE carcinomas. Of these 50%-60% are functionally active, i.e. due to largely uncontrolled secretion of insulin, gastrin, vasoactive intestinal polypeptide (VIP), glucagon or other even rarer hormones, such as adrenocorticotropic hormone (ACTH) or growth hormone (GH), they can induce characteristic syndromes (hypoglycemia syndrome, ZES, Verner-Morrison syndrome, glucagonoma syndrome, Cushing's syndrome, acromegaly)[23]. Depending on the predominant hormone secreted, these tumors are referred to as insulinomas, gastrinomas, VIPomas, glucagonomas, etc. Pancreatic endocrine tumors without hormonal symptoms are observed more frequently than previously, though this probably does not reflect an actual increase in frequency, but rather improved diagnostic methods and an increased resection rate. These nonfunctioning NETs are either incidental findings or are discovered along with general neoplastic symptoms, usually, however, because of local symptoms.

Pancreatic neuroendocrine tumors are extremely rare in childhood, but in adults they occur in all age groups and in males and females with equal frequency. Macroscopically, they are well demarcated, usually solitary, round tumors with a diameter measuring between 1 and 4 cm, and they can occur in all parts of the pancreas. In case of a hormonal syndrome, the hormone causing the syndrome can be detected immunocytochemically.

Although the pancreatic NETs are histologically well differentiated, they are frequently malignant, with the exception of insulinomas. This holds particularly for gastrinomas, VIPomas, glucagonomas and nonfunctioning tumors. The most important criteria of malignancy are, apart from metastases to the regional lymph nodes and the liver or invasion of adjacent organs, a tumor size of more than

*Table 5. Classification of neuroendocrine tumors of the ileum, cecum, colon and rectum*

1. Well-differentiated neuroendocrine tumor (carcinoid)
   - Benign: nonfunctioning, confined to mucosa-submucosa, nonangioinvasive, ≤ 1 cm (ileum) or ≤ 2 cm (colon-rectum)
     – Serotonin-producing tumor
     – Enteroglucagon-producing tumor
   - Benign or low grade malignant (uncertain malignant
     – potential): nonfunctioning, confined to mucosa-submucosa, angioinvasive, or <1 cm (ileum) or <2 cm (colon-rectum)
     – Serotonin-producing tumor
     – Enteroglucagon-producing tumor
2. Well-differentiated neuroendocrine carcinoma (malignant carcinoid)
   – Low grade malignant: invasion of the muscularis propria or beyond, or metastases
   – Nonfunctioning or functioning serotonin-producing carcinoma (with carcinoid syndrome)
   – Nonfunctioning enteroglucagon-producing carcinoma
3. Poorly differentiated neuroendocrine carcinoma · High grade malignant

*Table 6. Classification of neuroendocrine tumors of the pancreas*

1. Well-differentiated neuroendocrine tumor
   - Benign: confined to pancreas, < 2 cm in size, nonangioinvasive, ≤ 2 mitoses/HPF and ≤ 2% Ki-67-positive cells
     – Functioning: insulinoma
     – Nonfunctioning
   - Benign or low grade malignant (uncertain malignant potential): confined to pancreas, ≥2 cm in size, >2 mitoses/HPF, >2% Ki-67-positive cells, or angioinvasive
     – Functioning: gastrinoma, insulinoma, VIPoma, glucagonoma, somatostatinoma, or ectopic hormonal syndrome
     – Nonfunctioning
2. Well-differentiated neuroendocrine carcinoma
   - Low grade malignant: invasion of adjacent organs and/or metastases
     – Functioning: gastrinoma, insulinoma, glucagonoma, VIPoma, somatostatinoma or ectopic hormonal syndrome
     – Nonfunctioning
3. Poorly differentiated neuroendocrine carcinoma · High grade malignant

*Table 7. Criteria for assessing the prognosis of neuroendocrine tumors of the gastrointestinal tract*

| Biological behavior | Metastases | Invasion of m. propria* | Histological differentiation | Tumor size | Angioinvasion index | Ki-67 syndrome | Hormonal |
|---|---|---|---|---|---|---|---|
| Benign | – | – | Well differentiated | ≤1 cm* | – | <2% | –* |
| Benign or low grade malignant | – | – | Well differentiated | ≤2 cm | –/+ | <2% | – |
| Low grade malignant | + | +# | Well differentiated | >2 cm | + | >2% | + |
| High grade malignant | + | + | Poorly differentiated | Any | + | >30% | – |

* Exception: malignant duodenal gastrinomas are usually smaller than 1 cm and confined to the submucosa
# Exception: benign NETs of the appendix usually invade the muscularis propria

2 cm, angioinvasion and proliferative activity of more than 2%[10,24]. Recent studies have shown that angioinvasion plays a more important role than was assumed in the classification presented here[25]. It is therefore recommended that a tumor be considered malignant if the presence of angioinvasion has been verified, even if no other criteria of malignancy are present.

Among the functioning tumors, insulinomas are most frequent. 95% of them are between 1 cm and 2 cm in size and benign. Multiple insulinomas and insulinomas associated with MEN-1 are observed in approx. 10% of the cases. Compared with solitary or sporadic insulinomas, their rate of malignancy is not increased. Histologically, in approx. 5% of insulinomas amyloid deposits are found in the tumor. Immunocytochemically, insulin and proinsulin can be detected in varying ratios, depending on the grade of differentiation of the tumor[26], which is also reflected in the secretory behavior[27].

Pancreatic gastrinomas are usually larger than 2 cm and therefore in 60% of the cases they have already metastasized at the time of diagnosis. In contrast to duodenal gastrinomas, they rarely occur in the context of MEN-1[20].

Included among the rare tumors with hormonal syndromes are VIPomas, glucagonomas, ACTH-producing tumors and growth hormone-producing tumors. At the time of diagnosis most of these tumors are larger than 2 cm and have already metastasized. This is also true of nonfunctioning tumors.

Table 6 shows the prognostically relevant classification of the NET of the pancreas with all of the above described entities and peculiarities.

## Conclusions

The high degree of morphological and biological heterogeneity of the "carcinoids" has made it necessary to introduce a new generic term for them. In the WHO classification of endocrine tumors the terms neuroendocrine tumor (NET) and neuroendocrine carcinoma are therefore used. The 2000 WHO classification of gastroenteropancre-

*Table 8: Criteria for assessing the prognosis of neuroendocrine tumors of the pancreas*

| Biological behavior | Metastases | Invasion* | Histological differentiation | Tumor size | Angioinvasion | Ki-67 index | Hormonal syndrome |
|---|---|---|---|---|---|---|---|
| Benign | – | – | Well differentiated | ≤1cm | – | <2% | –/+# |
| Benign or low grade malignant | – | – | Well differentiated | >2cm | –/+ | <2% | –/+° |
| Low grade malignant | + | + | Well differentiated | >3cm | + | >2% | +° |
| High grade malignant | + | + | Poorly differentiated | Any | + | >30% | – |

* Invasion of adjacent organs (e.g. duodenum, stomach), # insulinomas, ° insulinomas and other functioning tumors (e.g. glucagonomas)

atic NETs (GEP-NETs), which we have commented on and summarized, provides a clinically relevant registry of the localization, biology and prognosis of individual NETs. For the practical diagnosis of GEP-NETs the criteria listed in Tables 7 and 8 can be used as a checklist with which to appropriately classify an individual tumor. Naturally this catalog of criteria needs to be extended and refined, because especially the group of NETs of questionable malignant potential ("benign or malignant behavior possible") is too large to be accepted in the long run in this indefinite form. Since the traditional morphological criteria have largely been exhausted, intensive work is currently being done on the molecular characterization and differentiation of the GEP-NETs. These studies have identified the gene responsible for MEN-1 on chromosome 11q13, which is also mutated in up to 40% of sporadic GEP-NETs[28]. By means of studies of comparative genomic hybridization (CGH) and allelic loss (LOH) a large number of genomic regions with loss or gain of genetic material have been detected[29, 30]. In this manner changes are being identified that may play a role in tumor progression. Such studies have also confirmed that NETs in different localizations are genetically independent tumors. Hence foregut NETs often show loss of 11q. This distinguishes them from NETs of the mid- and hindgut[31], which frequently show losses on chromosome 18q[32, 33]. On the basis of these results an intensive search is currently under way for genetic markers or marker constellations that will enable us to better predict the biological behavior of the group of GEP-NETs of uncertain malignant potential.

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Sažetak

## OD KARCINOIDA DO BIOLOŠKI I PROGNOSTIČKI ZNAČAJNE KLASIFIKACIJE NEUROENDOKRINIH TUMORA PROBAVNOG TRAKTA I GUŠTERAČE

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Iako je dobro poznat u medicinskoj terminologiji, pojam karcinoid nije više dovoljan da bi pokrio čitav morfološki i biološki spektar neoplazma diseminiranog neuroendokrinog staničnog sustava. Stoga se u klasifikaciji što ju je 2000. godine objavila SZO umjesto 'karcinoidi' rabe opći pojmovi 'neuroendokrini tumor' i 'neuroendokrini karcinom'. U ovom preglednom članku opisujemo klasifikaciju gastroenteropankreatičnih neuroendokrinih tumora, koja se temelji na kriterijima SZO. Također dajemo klasifikaciju i primjerene napomene o najvažnijim tumorskim entitetima. Na osnovi lokalizacije i različitih morfoloških i bioloških kriterija razlikujemo benigne neuroendokrine tumore, tumore neodređenog malignog potencijala, te tumore koji pokazuju nizak i visok stupanj malignosti.

Ključne riječi: *karcinoidi, neuroendokrini tumori, klasifikacija, prognoza, dijagnostika*