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REVIEW ARTICLE

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Revised classification of neuroendocrine tumours of the lung, pancreas and gut

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Abstract In this article new classifications of the neuroendocrine tumours of the lung, pancreas and gut are proposed. These classifications use a common frame work and attempt to consider the morphological, functional as well as biological features of the tumours.

Key words Neuroendocrine tumour · Classification
Lung · Pancreas · Gut

Introduction

Endocrine tumours arising in the lung, gut and some other places are collectively termed carcinoids. However, carcinoids are heterogeneous regarding histological differentiation, hormone production and biology. Moreover, tumours lacking the features of carcionids have also been found to be endocrine in nature. The use of the term carcinoid has therefore become increasingly difficult. This stimulated us to propose a new classification of the endocrine tumours of the lung, gut and pancreas. As all these tumours share the features of the neuroendocrine cell system, a short definition of the neuroendocrine cells, their characteristics and most important markers, precedes the sections concerned with the classification of the tumours.

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Definition of the neuroendocrine cell system

The term "neuroendocrine" is used here to define cells by their secretory products and some cytoplasmic proteins rather than their localization and embryological derivation. The neuroendocrine cell system therefore includes all neuronal and endocrine cells which share a common phenotype characterized by simultaneous expression of certain marker proteins (general neuroendocrine markers) and cell type specific hormonal products. The neuroendocrine cells form either small organs (i.e. anterior pituitary), distinct cell clusters within other tissues (i.e. pancreatic islets) or a network of cells dispersed in the thymus, thyroid, lung and gut. The tumours originating from these cells are rare but, by reproducing many of the functional features of their cell(s) of origin, may give rise to characteristic clinical syndromes [30, 94].

General features of neuroendocrine cells

Neuroendocrine cells have uniform nuclei and abundant granular or faintly staining (clear) cytoplasm. If they are gland-forming they present in solid clusters or show a trabecular pattern. If they are dispersed among other cells they are often difficult to recognize with the light microscope. Among the staining techniques which help to identify these cells the most important are the argentaffin and argyrophilic methods. At the ultrastructural level, they are characterized by the presence of membrane bound, dense-core secretory granules (diameter >80 nm) in the cytoplasm. They also contain small clear vesicles, (diameter 40–80 nm) which correspond to the synaptic vesicles of neurons.

General neuroendocrine markers

During the last 15 years a number of components of neuronal and neuroendocrine cells have been identified that

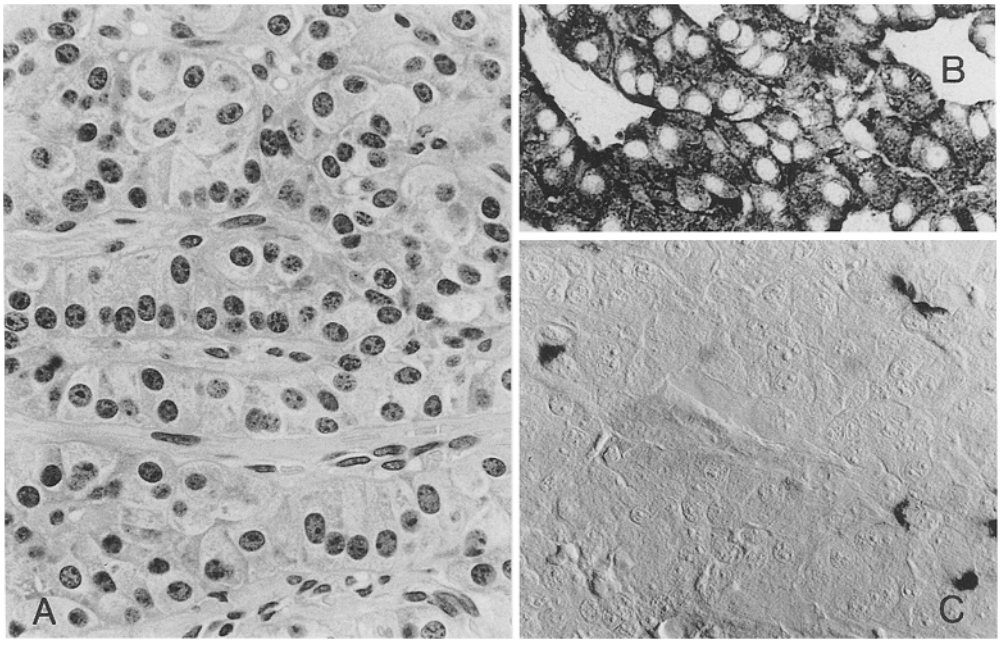


Fig. 1 Well differentiated non-functioning neuroendocrine tumour of the pancreas showing (**a**) a mixed solid-trabecular pattern with (**b**) intense immunostaining for synaptophysin and (**c**) little immunostaining for chromogranin A. ×250

serve as general markers [57, 94, 128]. The importance of general markers is to provide the pathologist with a clue to the neuroendocrine phenotype of a tumour, if not previously revealed by conventional histology. In addition, the significance of general markers is their independency of hormone production by tumour cells. They are therefore very valuable in the diagnosis of tumours not producing and/or secreting specific messengers and are used in a first step of a diagnostic algorithm for neuroendocrine tumours. These markers are associated with cytosolic proteins, small secretory vesicles or dense-core secretory granules [15, 61].

Cytosolic markers

Neuron specific enolase (NSE), the gamma-gamma dimer of the glycolytic enzyme enolase, is the best known marker of cells with neuroendocrine differentiation [68, 117]. The advantage of NSE as a marker is that its reactivity is unrelated to the content of secretory granules in the cells. Its disadvantage is that it may also stain some nonneuroendocrine tumour tissues such as solid-cystic (papillary-cystic) tumour of the pancreas [25], Schwannoma, carcinoma and fibroadenoma of the breast, renal cell carcinoma, chordoma, giant cell tumour of the tendon sheet [124] and certain malignant lymphomas [84]. These findings therefore advise caution in using NSE positivity as a neuroendocrine marker reaction in tumour diagnosis.

Protein gene product 9.5 (PGP 9.5) is a cytoplasmic protease originally extracted from brain tissue and found to be a marker of neuronal cells as well as neuroendocrine cells. It is biochemically and immunologically entirely different from NSE [119]. Its function is unknown. PGP 9.5 occurs in neuroendocrine neoplasms, including pancreatic endocrine tumours [99], neuronal tumours and most melanomas. It frequently co-localizes with NSE and, like NSE, diffusely stains the cytoplasm and the nucleus. Among the nonneuroendocrine neoplasms that may stain for PGP 9.5 are adenocarcinomas of the lung [99].

7B2 is the code name of a 179 amino acid protein extracted from pig and human pituitary. It has been localized to several tissues including hypothalamus, pituitary, adrenal, thyroid, gut and endocrine pancreas [115]. In the pancreas 7B2 is largely confined to the B-cells [12]. The staining pattern for 7B2 in tumours is patchy and appears to be unrelated to NSE or PGP 9.5 positivity [7].

Small vesicle associated markers

Synaptophysin is an integral membrane glycoprotein of 38000 MW that occurs in presynaptic vesicles of neurons and small clear vesicles of normal and neoplastic neuroendocrine cells [18, 127, 128]. It is expressed independently of the other neuroendocrine markers, notably secretory granule products (Fig. 1a). Recently synapsin, synaptotagmin, SV2 or synaptobrevin have been identified as further components of the small clear vesicles [94].

Secretory granule associated markers

Chromogranins A, B and C (secretogranin II) are a group of acidic monomeric proteins of various sizes. They constitute a family of soluble proteins localized to the matrix of secretory granules of many neuroendocrine

cells [68, 97, 125, 128]. Chromogranin A is probably the precursor of pancreastatin with which it shows structural homology. The chromogranins serve as powerful universal markers for neuroendocrine tissues and tumours. The result of the immunostaining for chromogranins depends on the cell type and usually also on the number of secretory granules present in the cells (Fig. 1b). The secretory protein HISL-19 is a novel marker of neuroendocrine cells [64]. It is distinct from the chromogranins.

The marker Leu7 (HNK1, CD57) is characterized by a monoclonal antibody raised against a human T-cell leukaemia cell line. This antibody recognizes an epitope present on the cell membrane of natural killer cells in peripheral blood. The same epitope is also detected on myelin associated glycoprotein in the central and peripheral nervous system and intracellular proteins localized to secretory granules of a number of neuroendocrine cells [121].

Cell specific markers

The various cell types of the neuroendocrine cell system are identified by their specific cellular products such as peptides and biogenic amines usually acting as hormones or neurotransmitters. By using in situ hybridization their expression can be traced back to the gene level.

Terminology and classification of neuroendocrine tumours

Oberndorfer [85] introduced the term "carcinoid" in 1907 in order to describe a group of ileal tumours with a distinct clinical evolution and morphology. In the years 1952 to 1954 the association of these tumours with what is now known as "carcinoid syndrome", i.e. cutaneous flushing, diarrhoea, bronchoconstriction, and heart valve lesions [23], was recognised and the causative hormones identified [2, 38, 55, 66].

In 1963, Williams and Sandler [130] classified gut endocrine tumours into foregut (stomach, pancreas, duodenum and upper jejunum), midgut (lower jejunum, ileum, appendix, caecum) and hindgut (colon, rectum) tumours, with considerable clinico-pathological differences amongst the three groups. In the case of foregut tumours, the usefulness of such a classification in practical diagnostic work is, however, limited by its failure to characterize individual tumour entities with well defined histological, cytological, hormonal and/or clinicopathological profiles. In addition, lung, thymus and pancreatic endocrine tumours are better separated from those arising in the gastrointestinal tract, owing to their distinctive morphological, functional and clinical patterns.

In 1980, the WHO classification of endocrine tumours applied the term carcinoid to all tumours of the diffuse neuroendocrine system, excluding pancreatic endocrine tumour (islet cell tumour), medullary carcinoma of the thyroid, paraganglioma, small cell lung carcinoma and Merkel cell tumour of the skin. The carcinoids were then subdivided on the basis of various silver and other granule staining techniques in (i)enterochromaffin (EC) cell carcinoids ("classical" carcinoids, "argentaffinomas"), (ii) gastrin (G) cell carcinoids and (iii) other carcinoids. The broad use of the WHO terminology, however, has proved difficult and often created confusion among pathologists and clinicians. This is primarily due to the fact that the wide application of progressively refined techniques in pathology, i.e. the progress from hematoxylin-eosin stain to the methods of biochemistry, histochemistry, immunocytochemistry and molecular biological techniques, has revealed a great diversity among neuroendocrine tumours. Thus tumours were identified as neuroendocrine neoplasms which lacked the histological characteristics of endocrine differentiation but displayed neuroendocrine features when examined immunocytochemically. The historical term "carcinoid" has therefore become more and more inappropriate to encompass all neoplasms with neuroendocrine features [31]. Secondly, the clinically characterized carcinoid syndrome only relates to a certain type of carcinoid, the EC-cell carcinoid which produces serotonin and substance P, while non-EC cell carcinoids are associated either with other endocrine syndromes or are functionally silent. Thirdly, there is a great deal of uncertainty among pathologists concerning the prognosis of the various "carcinoids" because the WHO classification vaguely considers the biological behaviour of the neuroendocrine neoplasms.

Based on earlier suggestions [32, 46, 92] we therefore propose to replace the term "carcinoid" by the term "neuroendocrine tumour" for the designation of the totality of neoplasms with neuroendocrine features. The term neuroendocrine tumour hence includes the entire neuroendocrine tumour spectrum ranging from classical carcinoids with slow growth and relatively good prognosis to very malignant undifferentiated carcinomas.

A further objective of this article is to propose neuroendocrine tumour classifications which follow comparable guidelines. The first principle is that the tumours are distinguished according to the site of origin. Here we consider the neuroendocrine tumours of the lung, pancreas, stomach, duodenum, jejunum and ileum, as well as colon and rectum. The neoplasms arising at these primary sites are subdivided into tumours with benign behaviour, tumours with uncertain behaviour (i.e. which may behave benign or become low-grade malignancies), low grade malignancies and finally highly malignant neoplasms. The criteria for these separations are the tumours' histological differentiation, size, extension into surrounding tissues, and angioinvasion (Fig. 2). As it has been shown that hormonal function and clinical associations of the neuroendocrine tumours are closely related to their clinical behaviour, these criteria have also been considered. For the sake of prognostic evaluation the term "functioning" is restricted here to those tumours which cause a clinical syndrome of endocrine hyperfunction in addition to inappropriately elevated serum hormone concentrations.

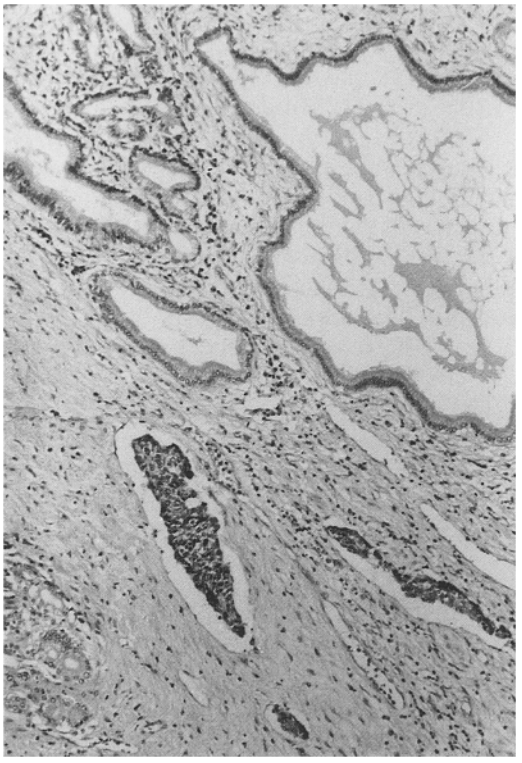


Fig. 2 Pancreatic neuroendocrine carcinoma showing angioinvasion. H & E, ×120

The new classification does not include neoplasms which have been termed mixed exocrine -endocrine or amphicrine tumours. Such tumours which exhibit multiple lines of divergent differentiation have first been described in the appendix and called goblet-cell carcinoids. Recently, adenocarcinomas from different locations showing neuroendocrine cells have been reported in increasing number. These observations have given rise to a confusing array of names. In future we should therefore attempt to define a more simple classification which separates truly mixed tumours, i.e. tumours in which the exocrine and endocrine cell populations are intimately mixed and the endocrine cell comprises about one half of the tumour tissue, from tumours with a diffusely scattered subpopulation of neoplastic neuroendocrine cells and collison tumours. So far it seems that these are only very few tumours qualifying as truly mixed, while the majority of so called mixed neoplasms only contains a small subpopulation of neuroendocrine cells. In general, mixed exocrine-endocrine neoplasms should be kept separate from neuroendocrine tumours, because the biological behaviour of the former tumours appears to be dictated by the differentiation of the exocrine cell compartment.

Lung tumours

The classification of neuroendocrine tumours of the lung as proposed here (Table 1) is based on the works of Arrigoni et al. 1972 [5], Gould et al. 1983 [47], Travis et al. 1991 [122] and on the recent comprehensive study of Bonato et al. 1992 [14]. The different types of neuroendocrine tumours of the lung are histologically defined, without use of immunocytochemistry (except well differentiated neuroendocrine carcinoma). Immunocytochemical detection of general neuroendocrine markers and specific peptides/amine is helpful, but without prognostic significance.

Table 1 Neuroendocrine tumours of the lung

Benign or low-grade malignant
Nonfunctioning well-differentiated tumour (typical carcinoid)
Functioning well-differentiated tumour
Low-grade malignant[a]
Nonfunctioning well-differentiated carcinoma (atypical carcinoid)
Functioning well-differentiated carcinoma
High-grade malignant
Functioning or nonfunctioning poorly differentiated carcinoma
Large cell type
Small or intermediate cell type

[a] If metastases or gross invasion are present, tumour should be called low-grade neuroendocrine carcinoma

Well differentiated neuroendocrine tumour, the classical carcinoid, represents one end of the spectrum of neuroendocrine tumours of the lung. It has an excellent prognosis after radical surgical treatment (90–98% 5-year survival). The diameter of these tumours is usually below 3 cm. The histological growth pattern can very from adenoid, solid, trabecular, paraganglioid to spindle cell-like, but has no influence on the biological behaviour. There is no or only a minor degree of cellular atypia, no necrosis, and no or very few (0–3/10 HPF) mitoses. S-100 positive satellite cells are often found within the tumours, particularly in those with solid and paraganglioid growth patterns.

Well differentiated neuroendocrine carcinoma, also known as atypical carcinoid, represents a tumour with a 60% 5-year survival rate after radical surgical treatment. There is a minor or moderate degree of cellular atypia, single cell necrosis or necrosis of small groups of tumour cells, and mitotic activity (3–10/10 HPF). Tumour cells do not show signs of crushing or nuclear moulding which are characteristic for small cell carcinoma. S-100 positive cells are rare, and often completely absent within the tumour.

Helpful prognostic indicators for well differentiated neuroendocrine tumour ("typical carcinoid") and well differentiated neuroendocrine carcinoma are lymph node metastasis (5-y survival for NO=89–95%; for N1=37%) and tumour size (5-y survival for <2 cm: 97%, >2 cm: 49%). Additional potential prognostic parameters including ploidy and S phase fraction [37], immunocytotochemical proliferation markers [9, 13], p53 [9, 69], expression of cell adhesion molecules [63] and apoptotic rate are currently under evaluation, but to date without clear significance or with contradictory results.

Neuroendocrine tumours of the lung representing high grade malignancies are either of the small cell/intermediate cell type or of the large cell type. While the diagnosis of the small/intermediate cell type is usually straight forward, the adequate diagnosis of the large cell type requires immunocytochemical demonstration of neuroendocrine markers in order to rule out large cell non-endocrine carcinoma. The 5-year survival rate after radical surgical treatment for stage I tumours was most recently reported between 37–55% [48]. Tumour cells are above 33 μm (=3 lymphocytes) in diameter, with vesicular chromatin, large distinct nucleoli, and moderate to severe atypia. The mitosis count is usually above 10/10HPF.

Pancreas tumours

The classification of the neuroendocrine tumours of the pancreas is based on the studies of Heitz 1984 [56]; Nauk and Creutzfeld 1991 [82]; Solcia et al. 1991 [106]; Stamm et al. 1991 [111]; Klöppel et al. 1993 [62]. According to these studies both the differentiation grade (in conventional histologic preparations) and the functional lineage (by hormone immunohistochemistry) of tumour cells should be assessed for appropriate prognostic evaluation of pancreatic endocrine tumours (Table 2).

The benign behaviour of well differentiated endocrine tumours of the pancreas less than 2 cm in size and noninvasive, either nonfunctioning or causing the insulinoma syndrome, is supported by many studies [56, 62, 106, 110, 112]. More than 50% of tumours causing the gastrinoma, vipoma, glucagonoma, Cushing or carcinoid syndromes have been shown to be malignant (i.e. grossly invasive or metastatic). At diagnosis most of these tumours were larger than 2 cm [20, 36, 50, 101, 111]. Thus, whenever exceeding 2 cm in size or angioinvasive, they are to be classified among tumours of low grade malignancy. Insulinomas and nonfunctioning tumours have a high rate of malignant behaviour only when above 3 cm in size or when unquestionably angioinvasive [27, 28, 60, 62, 106]. Well differentiated noninvasive insulinomas or nonfunctioning endocrine tumours ranging from 2 and 3 cm and the other functioning tumours measuring between 1 and 2 cm form a group of tumours the behaviour of which is difficult to predict in the individual case, although the majority of them, when followed, show benign behaviour [67, 82, 106]. Aneuploidy with a DNA index greater than 1.5, more than 5 mitoses/10 HPF, more than 5% AgNOR rich cells, a PCNA index above 5% and expression of human chrorionic gonadotropin alpha [34, 90, 100] have all been suggested to predict malignancy, but still await confirmation or better definition. When the malignancy of a well differentiated tumour is proven at first diagnosis by metastasis or gross local invasion, it should be called neuroendocrine carcinoma, low grade.

Poorly differentiated neuroendocrine carcinomas of the pancreas are mainly composed of intermediate rather than small cells (Fig. 3) and, like comparable tumours from other sites, have very poor prognosis and only occasionally may sustain a hyperfunctional syndrome [26, 87, 95]. Most patients die with wide-spread metastatic disease within one year from diagnosis.

Stomach tumours

The classification of the neuroendocrine tumours of the stomach is based on the studies of Carney et al. 1983

Table 2 Neuroendocrine tumours of the pancreas

Benign	
Functioning well-differentiated non-angioinvasive tumour of small size	
insulinoma	(size <2 cm)
others[b]	(size <1 cm)
Nonfunctioning well-differentiated non-angioinvasive tumour of small size	(<2 cm)
Benign or low-grade malignant	
Functioning well-differentiated non-angioinvasive tumour of intermediate size	
insulinoma	(size 2–3 cm)
others[b]	(size 1–2 cm)
Nonfunctioning well-differentiated non-angioinvasive tumour of intermediate size (2–3 cm)	
Low-grade malignant[a]	
Functioning well-differentiated tumour of large size and/or with angioinvasion	
insulinoma	(size >3 cm)
others[b]	(size >2 cm)
Nonfunctioning well-differentiated tumour of large size (>3 cm) and/or with angioinvasion	
High-grade malignant	
Functioning or nonfunctioning poorly differentiated intermediate or small cell carcinoma	

[a] If metastases or gross invasion are present, tumour should be called low-grade neuroendocrine carcinoma

[b] Other functioning tumours: gastrinoma, vipoma, glucagonoma, serotonin producing tumour, others

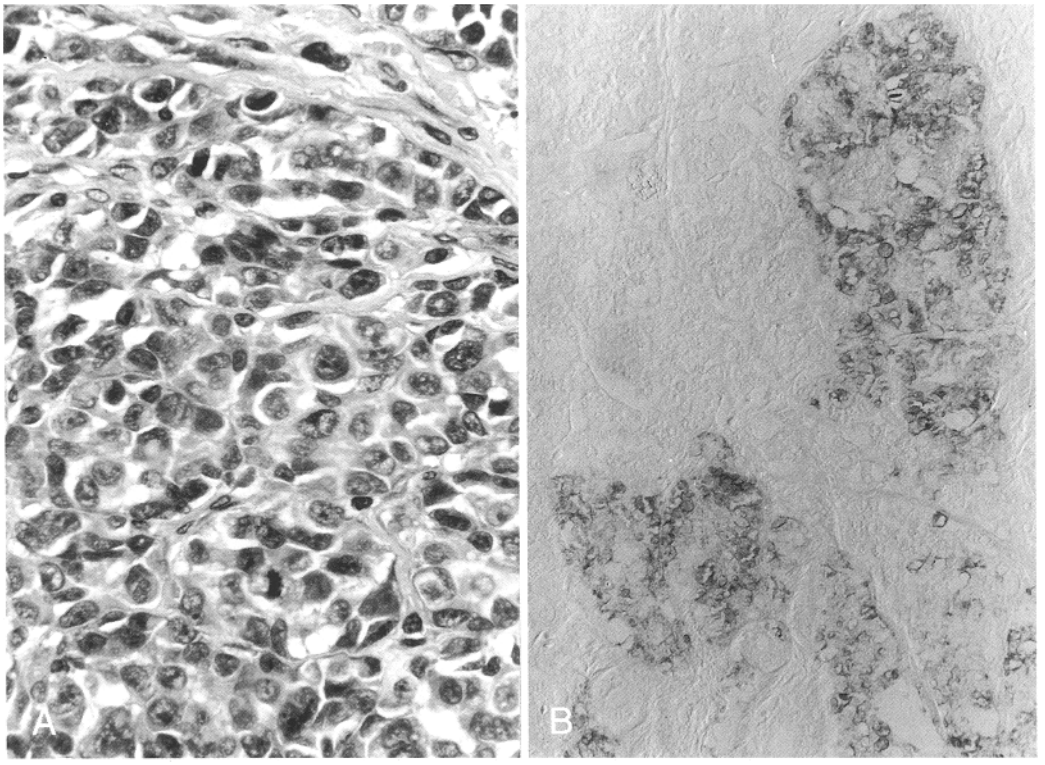


Fig. 3 Poorly differentiated nonfunctioning neuroendocrine carcinoma of the pancreas. (**a**) The tumour consists of small to intermediate sized cells and (**b**) stains for synaptophysin. ×250

Table 3 Neuroendocrine tumours of the stomach

Benign
Nonfunctioning well-differentiated tumour of small size (up to 1 cm) within the mucosa-submucosa and without angioinvasion
– usually ECL-cell tumours of the fundic mucosa associated with chronic atrophic gastritis (CAG) and hypergastrinaemia
Benign or low-grade malignant
Nonfunctioning well-differentiated tumour within the mucosa-submucosa of intermediate size (>1 up to 2 cm) without angioinvasion or of small to intermediate size (up to 2 cm) with angioinvasion
– usually ECL-cell tumours of the fundic mucosa associated with CAG and hypergastrinaemia
– rarely MEN-1 associated or sporadic ECL-cell tumours
Low-grade malignant[a]
Nonfunctioning well-differentiated tumour of large size (>2 cm) or extending beyond the submucosa
– usually sporadic ECL-cell tumours; rarely serotonin producing tumours[b] or others
– rarely MEN-1 or CAG associated ECL-cell tumours
Functioning well-differentiated tumour of any size and extension
– sporadic gastrinoma, serotonin producing tumour[b] or others
High-grade malignant
Functioning or nonfunctioning poorly differentiated intermediate or small cell carcinoma

[a] If metastases or gross invasion are present, tumour should be called low-grade neuroendocrine carcinoma
[b] Also called EC-cell tumour

[22], Solcia et al. 1988 [104] and Solcia et al. 1990 [105], Stolte et al. 1988 [114], Bordi et al. 1991 [16], Matsui et al. 1991 [74] and Rindi et al. 1993 [98] (Table 3).

Separation of well differentiated neuroendocrine microtumours (or "microcarcinoids") from their precursor (precarcinoid) lesions has been proposed at 0.5 mm size [104]. The finding of such "precursor" lesions in a biopsy should encourage a search for possibly coexisting microtumours difficult to detect at endoscopy. The benign behaviour of nonfunctioning well differentiated neuroendocrine tumours (or "carcinoids") measuring up to 1 cm in size, confined to the mucosa or with infiltration limited to the muscularis mucosae and devoid of frank cytologic atypia, is documented by several clinico-pathologic studies [16, 22, 53, 98, 114]. Most of them are mainly composed of argyrophil ECL cells and arise as multiple growths (so called "carcinoidosis") in severely atrophic corpus-fundus mucosa due to autoimmune destruction of acidopeptic glands (diffuse atrophic gastritis restricted to the body-fundus), causing profound achlorhydria and secondary hypergastrinaemia, with or without pernicious anemia. In rare cases an end stage *Helicobacter pylori* gastritis (atrophic gastritis of the body-fundus with disseminated, surviving acidopeptic glands, with or without antral involvement) has been found [107]. Another, although rare, background condition favouring the development of multiple ECL cell tumours is combined multiple endocrine neoplasia type 1 (MEN-1) and Zollinger-Ellison syndrome (ZES). In this condition, hypergastrinaemia causes hypertrophic gastropathy with acido-peptic gland hypertrophy and argyrophil ECL cells hyperplasia, while the combined action of the MEN-1 and chronic hypergastrinaemia seems to induce ECL cell tumours [105].

Well differentiated neuroendocrine tumours arising sporadically in the gastric mucusa, independently from chronic atrophic gastritis, MEN-1/ZES and hypergastrinaemia, when more than 2 cm in size and/or showing angioinvasion or deep wall invasion, proved metastatic in more than 60% of cases, with liver metastases in about 50% of cases [98]. Mean survival of fatal cases was about 4 years. About one fourth showed a carcinoid syndrome, of either classic or "histamine" type [1]. The behaviour of well differentiated, nonfunctioning endocrine tumours, single or multiple, limited to mucosa-submucosa and between 1 and 2 cm, with or without associated atrophic gastritis, MEN-1/ZES and hypergastrinaemia, remains difficult to predict, although the majority of cases so far investigated showed localized disease [76, 98, 129].

Poorly differentiated neuroendocrine carcinomas of the stomach are mainly composed of intermediate rather than small size cells and, like comparable tumours from other sites, show very poor prognosis, with 3/4 of the patients dying within one year from diagnosis due to extensive metastatic disease [74, 98].

Duodenum tumours

The classification of the neuroendocrine tumours of the duodenum is based on the studies of Dayal et al. 1983 [29], Lasson et al. [65], Hamid et al. 1986 [52], Stephens et al. 1987 [113], Barabareschi et al. 1989 [8], Burke et al. 1989 [19], Pipeleers-Marichal et al. 1990 [92], Zamboni et al. 1990 [133], Capella et al. 1991 [21], Sanchez-Sosa et al. 1991 [103], Dookhan et al. 1993 [35], Ricci 1993 [96], and Hatzitheoklitos et al. 1994 [54]. According to these studies five major types of neuroendocrine tumours can be distinguished in the duodenum: Gastrin producing tumour, somatostatin producing tumour, gangliocytic paraganglioma, serotonin/calcitonin/PP-producing tumours, and poorly differentiated carcinoma.

Gastrin producing tumours are most frequent and represent about two third of all cases. They reside preferentially in the proximal duodenum and show a trabecular pattern. Although usually small (<1 cm), most of them, when functioning, have already metastasized to the regional lymph nodes at the time of diagnosis. These lymph node metastases may be much larger than the primaries in the duodenum [93]. Spread to the liver, however, is a rare and late event. Approximately one third of the tumours is associated with a ZES which may be part of the MEN-1 syndrome. If, vice versa, all patients with a sporadic ZES are considered, at least 40% of them appear to have a duodenal gastrinoma, while in the remaining patients the tumour is found in the pancreas. In MEN-1 patients with ZES, the incidence of duodenal gastrinoma is even higher and may reach 90%. Another characteristic of MEN-1 associated duodenal gastrinomas is their frequent multicentricity (Fig. 4). According to these features most functioning gastrin cell tumours fall in the category of neuroendocrine tumours of the du-

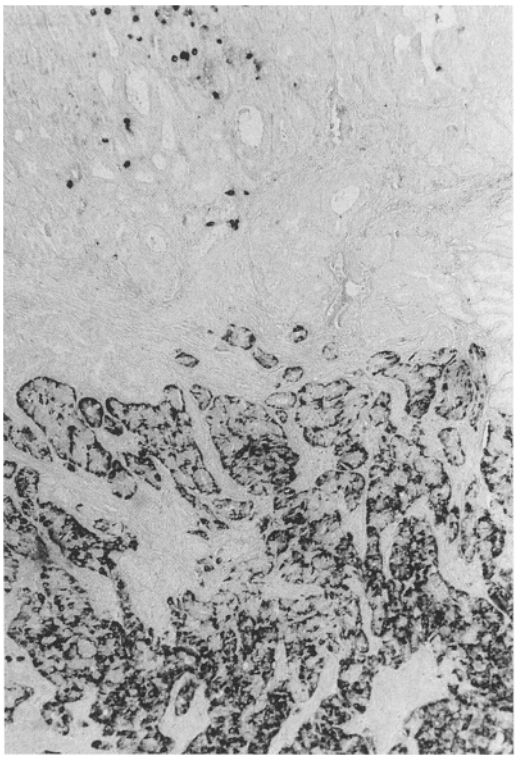


Fig. 4 Well differentiated, low-grade malignant neuroendocrine tumour of the duodenum in a patient with MEN-1 and Zollinger-Ellison syndrome. This tumour had already metastasized to regional lymph nodes. Immunostaining for gastrin. ×60

Table 4 Neuroendocrine tumours of the duodenum

Benign
Nonfunctioning well-differentiated tumour of small size (≤1 cm) within the mucosa-submucosa and without angioinvasion
– gastrin or serotonin[b] producing tumours in the proximal duodenum
Gangliocytic paraganglioma (any size; periampullary region)
Benign or low-grade malignant
Nonfunctioning well-differentiated tumour within the mucosa-submucosa,
of intermediate size (>1–2 cm) without angiovasion or of small to intermediate size (up to 2 cm) with angioinvasion
– serotonin producing[b] tumours or others (any site)
– somatostatin producing tumours (ampullary region) with or without Recklinghausen's disease
Low-grade malignant[a]
Nonfunctioning well-differentiated tumour of large size (>2 cm) or extending beyond submucosa
– gastrin or serotonin producing[b] tumours (any site)
– somatostatin producing tumours (ampullary region) with or without associated Recklinghausen's disease
Functioning well-differentiated tumour of any size and extension
– sporadic gastrinoma, serotonin producing tumour[b] or others
– hereditary MEN-1 associated gastrinoma, usually multiple
High-grade malignant
Functioning or nonfunctioning poorly differentiated intermediate or small cell carcinoma (usually ampullary region)

[a] If metastases or gross invasion are present, tumour should be called low-grade neuroendocrine carcinoma
[b] Also called EC-cell tumour

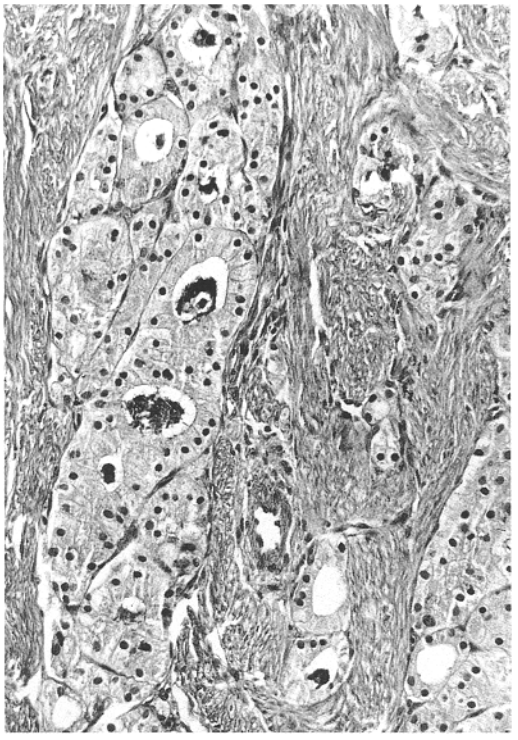


Fig. 5 Well differentiated low-grade malignant neuroendocrine tumour of the ampulla of Vater (which stained for somatostatin) showing a glandular pattern and psammoma bodies (*arrows*). H & E, ×120

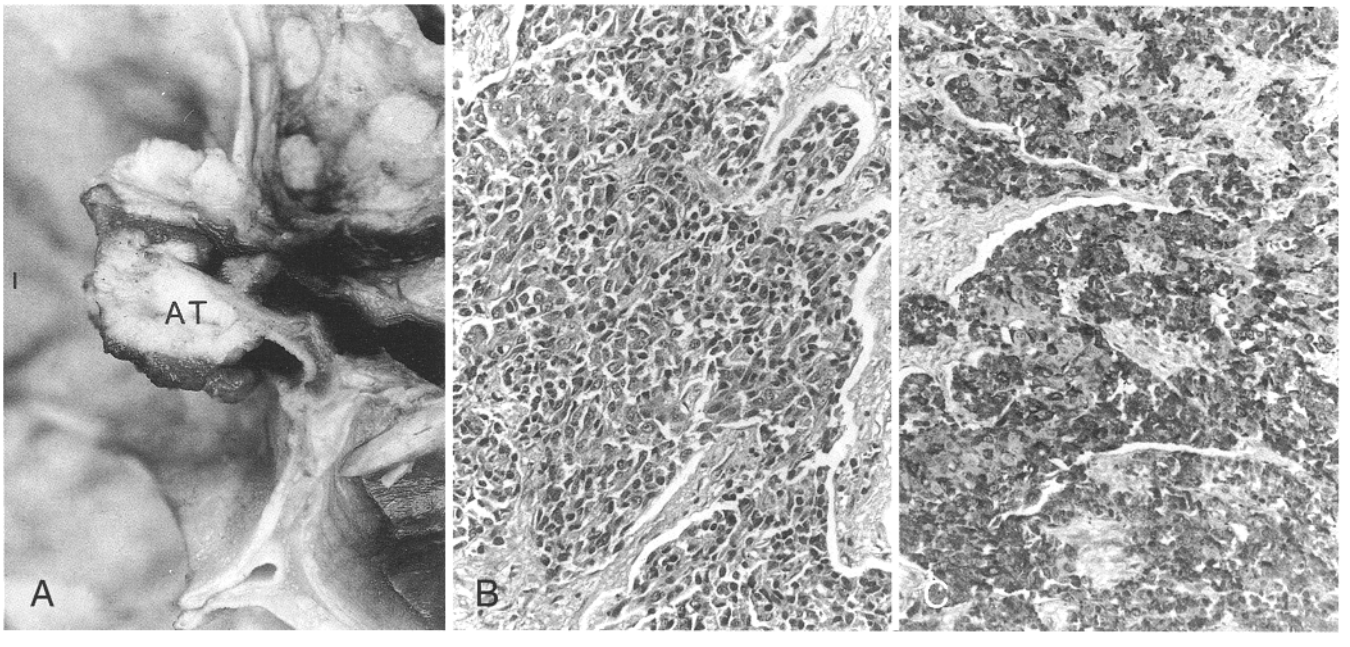
Fig. 6 Poorly differentiated malignant neuroendocrine tumour of the ampulla of Vater. (**a**) The small ampullary tumour (*AT*) gave rise to wide-spread metastasis. Note paraduodenal lymph node metastases. (**b**) The H & E shows intermediate sized cells (**c**) which stain for synaptophysin. ×120

odenum with low-grade malignant behaviour, either as sporadic gastrinoma or hereditary MEN-1 associated gastrinoma (Table 4). In contrast, most nonfunctioning gastrin producing tumours show a benign behaviour [21].

Somatostatin producing tumours are second in frequency and comprise about 15–20% of all neuroendocrine tumours of the duodenum. They occur almost exclusively in the ampulla of Vater and are usually malignant. Histologically, they are characterized by glandular structures and often psammoma bodies (Fig. 5). Although they stain strongly for somatostatin, they are nonfunctioning. However, about one third of them is associated with neurofibromatosis type 1 (von Recklinghausen's disease). According to these features most somatostatin cell tumours will fall into the category of nonfunctioning well differentiated neoplasms with low-grade malignant behaviour (Table 4).

Third in frequency are gangliocytic paragangliomas. They occur in the ampullary-periampullary region. Histologically they are characterized by their gangliocytic component; immunocytochemically they express predominantly somatostatin and PP. Although often of large size (>2 cm) and with involvement of the muscularis propria they usually behave benign. Most of the gangliocytic paragangliomas thus fall in the category of tumours with benign behaviour (Table 4).

Duodenal neuroendocrine tumours producing serotonin or other hormones such as calcitonin and PP are rare. Outside of the ampulla they are usually small and lack signs of infiltrative growth. The occurrence of a carcinoid syndrome is exceptional. Hence most of them represent nonfunctioning well-differentiated tumours with benign behaviour (Table 4). A few of these tumours are found in the ampulla and about half of these fall into the category of tumours with low-grade malignant behaviour (Table 4).



Poorly differentiated neuroendocrine carcinomas are extremely rare. Most of them occur in the ampulla of Vater (Fig. 6). Histologically they represent undifferentiated carcinomas of the intermediate or small cell type. The neuroendocrine nature of these highly malignant neoplasms is only revealed by their positivity for general neuroendocrine markers.

Jejunum and ileum tumours

The classification of the neuroendocrine tumours of the jejunum and ileum is based on the studies of Moertel et al. 1961 [77], Hajdu et al. 1974 [51], Martensson et al. 1983 [72], Solcia et al. 1993 [108]. According to these studies the vast majority of tumours are classical ileal carcinoids with production of serotonin and substance P. These tumours have also been called EC-cell carcinoids or argentaffinomas. Enteroglucagon/PP/PYY producing tumours are exceptionally rare [108] (Table 5).

Serotonin and substance P producing neuroendocrine tumours occur preferentially in the second part of the ileum. Histologically they reveal a typical insular pattern. If found at autopsy, they are usually small (up to 1 cm) and show no metastases. These tumours constitute the neoplasms with benign behaviour (Table 5). In surgical series which encompass the symptomatic cases, most tumours are larger than 1 cm and those above 2 cm in diameter are almost all malignant, i.e. presenting with regional lymph node metastases. In up to 40% there are multiple tumours. Approximately 20% of the malignant tumours are associated with a carcinoid syndrome, which implies that these patients already have liver metastases.

Table 5 Neuroendocrine tumours of the jejunum and ileum

Benign
Nonfunctioning well-differentiated tumour of small size (≤1 cm) within the mucosa-submucosa but without angioinvasion
– usually serotonin producing[b] tumours in the terminal ileum
Benign or low-grade malignant
Nonfunctioning well-differentiated tumours of intermediate size (>1 up to 2 cm) but without angioinvasion or extension beyond the submucosa
– usually serotonin producing[b] tumours of the terminal ileum
Low-grade malignant[a]
Nonfunctioning well-differentiated tumour of large size (>2 cm) or extending beyond the submucosa and/or angioinvasive
– usually serotonin producing[b] tumours of the terminal ileum
Functioning well-differentiated tumour of any size and extension
– serotonin producing tumour[b] with carcinoid syndrome[c]
– sporadic gastrinoma (upper jejunum)
High-grade malignant
Functioning or nonfunctioning poorly differentiated intermediate or small cell carcinoma

[a] If metastases or gross invasion are present, tumour should be called low-grade neuroendocrine carcinoma.
[b] Also called EC-cell tumour.
[c] As serotonin is metabolized and inactivated by the liver, serotonin secreting tumours of the gut only produce a syndrome if liver metastases are already present

These tumours comprise the category of low-grade malignancies (Table 5).

Jejunal tumours are very rare. Histologically they show a trabecular pattern. Immunocytochemically they rather stain for gastrin, or somatostatin than for serotonin. Poorly differentiated neuroendocrine carcinomas are exceedingly rare in the jejunum or ileum.

Appendix tumours

The classification of the neuroendocrine tumours of the appendix is based on the studies of Masson 1928 [73], Dische 1968 [33], Moertel et al. 1968 [78], Chejfec et al. 1988 [24], Iwafuchi et al. 1990 [58], Solcia et al. 1993 [109]. According to these studies the vast majority of tumours are EC cell argentaffin carcinoids producing serotonin and substance P, while a minority are non-argentaffin L cell tumours producing glicentin-related peptides (enteroglucagons) and PP-PYY [24, 109] (Table 6). The neuroendocrine cells of human appendiceal mucosa that may give rise to neuroendocrine tumours are morphologically and functionally heterogeneous [6, 58, 73, 89].

The first group of neuroendocrine tumours of the appendix, the EC cell tumours producing serotonin and substance P, show a typical insular pattern [24]. These tumours do not appear to derive from the intraepithelial endocrine cells but from subepithelial neuroendocrine complexes [70, 73]. This view is supported by the finding that S-100 protein positive Schwann-like (sustentacular) cells are found as an integral component of appendi-

Table 6 Neuroendocrine tumours of the appendix

Benign
Nonfunctioning well-differentiated tumour of small size (<2 cm) without extension into the mesoappendix
– usually serotonin producing[b] tumours at the tip of the appendix
– rarely enteroglucagon producing tumours[d]
Benign or low-grade malignant
Nonfunctioning well-differentiated tumour of large size (>2 cm) with extension into the mesoappendix
– usually serotonin producing[b] tumours at the tip of the appendix
– rarely enteroglucagon producing tumours[d]
Low-grade malignant[a]
Nonfunctioning well-differentiated tumour of large size (>3 cm), with deep invasion into the mesoappendix
– serotonin producing tumours[b]
Functioning well-differentiated tumour of any size and extension
– serotonin producing tumour[b] with carcinoid syndrome[c]
High-grade malignant
Functioning or nonfunctioning poorly differentiated intermediate or small cell carcinoma

[a] If metastases or gross invasion are present, tumour should be called low-grade neuroendocrine carcinoma
[b] also called EC-cell tumour
[c] as serotonin is metabolized and inactivated by the liver, serotonin secreting tumours of the gut only produce a syndrome if liver metastases are present
[d] also called L-cell tumours, producing glucagon-, PP- and PYY-related peptides

ceal EC cell tumours, while they are lacking in ileal and colonic EC cell tumours, which develop from EC cell of the mucosal crypts [70].

The second much less common group of appendiceal neuroendocrine tumours is composed of non-argentaffin L cell tumours producing glicentin related peptides (enteroglucagons) and PP/PYY and showing a trabecular growth pattern [58, 109].

Appendiceal neuroendocrine tumours have a very low aggressive behaviour. The reported frequency of metastasis from these tumours lies between 1.4% and 8.8% [4, 17, 43, 44, 79, 116, 118]. According to one study, in all cases of metastasis, the size of the primary tumour was greater than 2.0 cm [78]. In an analysis of 414 cases from the literature, Mc Gillivray et al. [71] found that both tumour size greater than 2 cm ($P<0.0001$) and invasion of the mesoappendix ($P<0.0001$) were related to the presence of metastasis in the entire group. In those patients with tumours smaller than 2 cm, mesoappendiceal invasion was significantly associated with metastasis ($P<0.0001$). Size of the tumours is then clearly related to the risk of malignant behaviour but cannot be relied on as the sole predictor of malignancy. Invasion of the mesoappendix is predictive of an increased risk of metastasis for carcinoid tumours of the appendix less than 2 cm [17, 71].

Location of the tumours at the base of the appendix with involvement of the surgical margin or of the caecum is an unfavourable prognostic feature which requires at least a partial caecectomy to avoid residual tumour or subsequent recurrence [120].

The carcinoid syndrome is very rarely observed in neuroendocrine tumours of the appendix. When it manifests, it is almost always associated with widespread metastases of the tumour, predominantly to the liver or retroperitoneum [43, 118].

Colon and rectum tumours

The classification of the neuroendocrine tumours of the colon and rectum is based on the studies of Berardi 1972 [11], Gould and Chejfec 1978 [45], Fiocca et al. 1980 [40], Alumets et al. 1981 [3], O'Briain et al. 1982 [86], Mills et al. 1983 [75], Wick et al. 1987 [126]. According to these studies at least three main types of neuroendocrine tumours have been identified: L cell tumours producing glicentin-related peptides and PP-PYY, EC cell tumours producing serotonin and substance P, and poorly differentiated (small cell) carcinomas (Table 7).

Well differentiated L cell tumours showing a trabecular pattern usually occur in the rectum, and only rarely in the colon [109]. Glucagon-29, glucagon-37, glicentin, proglucagon cryptic fragments, PYY, PP and pro-PP icosapeptide, all prove to be useful immunohistochemical markers of rectal L cell tumours [41]. Although in these tumours there is a prevalence of L cells, minor populations of serotonin, substance P, somatostatin, insulin, enkephalin, beta-endorphin, neurotensin, alpha-hCG and motilin immunoreactive cells have also been identified [39, 41, 42, 81, 131]. To date, there is only one report of a rectal trabecular neuroendocrine tumour in which motilin cells represented the prevailing cell population [49].

Table 7 Neuroendocrine tumours of the colon and rectum

Benign
Nonfunctioning well-differentiated tumour of small size (<2 cm) within the mucosa-submucosa and without angioinvasion
– trabecular enteroglucagon producing tumours[d] usually in the rectum
– serotonin producing tumours[b], usually in the caecum or colon
Benign or low-grade malignant
Nonfunctioning well-differentiated tumour of small size (<2 cm) within the mucosa-submucosa but with angioinvasion
– trabecular enteroglucagon producing tumours[d], usually in the rectum
– serotonin producing tumours[b], usually in the caecum or colon
Low-grade malignant[a]
Nonfunctioning well-differentiated tumour of large size (>2 cm), and/or extending beyond the submucosa
– trabecular enteroglucagon producing tumours[d], usually in the rectum
– serotonin producing tumours[b], usually in the caecum or colon
Functioning well-differentiated tumour of any size and extension
– serotonin producing tumour[b] with carcinoid syndrome[c]
High-grade malignant
Functioning or nonfunctioning poorly differentiated intermediate or small cell carcinoma

[a] If metastases or gross invasion are present, the tumour should be called low-grade neuroendocrine carcinoma
[b] Also called EC-cell tumour
[c] As serotonin is metabolized and inactivated by the liver, serotonin secreting tumours of the gut only produce a syndrome if liver metastases are present
[d] Also called L-cell tumours, producing glucagon-, PP- and PYY-related peptides

Argentaffin EC cell tumours, with characteristic insular pattern and serotonin production, are extremely rare in the rectum [132], while they represent the most frequent type of neuroendocrine tumours in the colon with prevalence for the caecum [11].

Colonic well differentiated neuroendocrine tumours usually are larger than well differentiated neuroendocrine tumours of the rectum. In the review of Berardi [11] the average size for colonic tumours with metastasis was 6.1 cm, while that of tumours without metastasis was 4.7 cm. In a series of 7 colonic neuroendocrine tumours Morgan et al. [80] found all tumours larger than 2 cm associated with lymph node metastases and 6 with distant metastases. As for colonic tumours, size is also the usual prognostic predictor of well differentiated neuroendocrine tumours of the rectum [80, 88]. Comparing "benign and malignant" carcinoids, Peskin and Orloff [91] found that nine of ten rectal carcinoids with diameters greater than 2 cm were locally invasive with or without distant metastases. On the other hand, 14 of 15 carcinoids measuring less than 2 cm in their largest diameters failed to show local invasion or metastasis. Bates

[10] confirmed the relationship between size and malignancy. Of 37 rectal neuroendocrine tumours which had metastasized 78% were larger than 2 cm in size. Only 8% of the 115 lesions smaller than 2 cm had developed metastases. The risk of a tumour smaller than 1 cm for mestastasizing is estimated at 3% or less [83]. A rectal neuroendocrine tumour between 1 and 1.9 cm in diameter carries a 7–11% risk of metastasizing [59, 83]. The presence of invasion of the muscularis propria has also been considered as an index of tumour aggressiveness [88]. For tumours of intermediate size (1-1.9), Naunheim et al. [83] found that 46% of lesions with invasion of the muscularis propria metastasized. The carcinoid syndrome was present in 4 of 118 cases of neuroendocrine tumours of the colon in Berardi's series [11] and in all these cases the tumours had metastases.

In a recent study determining the DNA content of 22 rectal carcinoids by cytophotometry of paraffin embedded tissues, it was shown that three of the tumours with synchronous or metachronous metastasis had aneuploid DNA pattern, whereas 19 tumours with no metastasis showed diploid DNA pattern, suggesting that DNA ploidy pattern may be a factor of significant prognostic value [123].

Poorly differentiated neuroendocrine carcinomas present as bulky rapidly developing masses [45, 102]. Their distribution in the colon-rectum resembles that of conventional adenocarcinomas. An origin of such neoplasms in conventional colonic adenomas has been described by Mills et al. [75]. By light microscopy the cells are small to intermediate in size, arranged in groups with extensive necrosis. Tumour cells are generally unreactive to antisera directed against hormonal peptides and amines, while they are strongly positive for both cytosolic neuroendocrine markers and epithelial antigens [126]. At the ultrastructural level, rarely occurring, small (100–250 μm) immature neurosecretory granules are observed [45, 126]. Clinically, there has been no hormonal syndrome in any of the cases reported so far. The prognosis is very poor and most patients die with widespread metastases within few months (average survival 5 months [126]) of clinical presentation.

Conclusions

In the last decade our knowledge and understanding of the neoplasms with neuroendocrine differentiation has tremendously increased. The classifications, however, used so far, only insufficiently consider all the newly established morphological, functional and biological features of these tumours. We therefore propose new classifications of the neuroendocrine tumours of the lung, pancreas, stomach, duodenum, jejunum and ileum, appendix, and colo-rectum. These classifications are based on a common frame work that subdivides the tumours in "benign", "benign or low-grade malignant", "low-grade malignant" and "malignant". We are aware that some uncertainties will remain, but hope that the use of these classifications in daily practice and their evaluation in clinicopathological studies will reveal that they more accurately reflect the biology of the neuroendocrine tumours than the so far available classifications.

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